THE TOXICITY OF CREOSOTE TREATED WOOD TO PACIFIC HERRING (*CLUPEA PALLASI*) EMBRYOS AND CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS NEAR CREOSOTED PILINGS IN JUNEAU, ALASKA

By

Danielle Duncan

RECOMMENDED:

Dr. Stanley Rice

Dr. Robert Perkins

Dr. Anthony Gharrett

Dr. Michael Stekoll, Advisory Committee Chair

Dr. Shannon Atkinson
Chair, Graduate Program, Fisheries Division

APPROVED:

Dr. Michael Castellini
Dean, School of Fisheries and Ocean Sciences

Dr. John Eichelberger
Dean of the Graduate School

8/25/14

Date

THE TOXICITY OF CREOSOTE TREATED WOOD TO PACIFIC HERRING (*CLUPEA PALLASI*) EMBRYOS AND CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS NEAR CREOSOTED PILINGS IN JUNEAU, ALASKA

A THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

Danielle Duncan, B.S.

Fairbanks, Alaska

August 2014

Abstract

These studies documented creosote toxicity to developing Pacific herring (*Clupea pallasi*) embryos at low microgram per liter concentrations and determined that detrimental concentrations of polycyclic aromatic hydrocarbons (PAHs) near creosoted pilings exist. Creosote total PAH concentrations of 7 µg/L resulted in skeletal defects and ineffective swimming in hatched larvae and represent a lowest observed effect concentration (LOEC) for Pacific herring embryos not previously defined. In the field, PAHs consistent with creosote were elevated at distances up to a meter from creosoted pilings in some cases. Concentrations likely sufficient to induce teratogenic effects were found directly on creosoted pilings and within ten centimeters of pilings. Cumulatively, these studies provide useful and needed data on the interactions between Pacific herring embryos and creosoted pilings in the nearshore environment.

Table of Contents

Page

List of Figures

Page

Preface and Acknowledgements

This thesis is the product of two projects both designed to better understand the ecotoxicological impact of creosote on developing Pacific herring embryos. The first project and chapter addresses the direct toxicity of creosote to Pacific herring embryos and was funded jointly by the Alaska Department of Transportation and Public Facilities and the University of Alaska Fairbanks Institute of Northern Engineering. The second project and chapter was funded by the National Marine Fisheries Service and explores environmental levels of hydrocarbons near creosoted pilings at harbors in Juneau, Alaska. The results of the creosote toxicity study have been reported to the Alaska Department of Transportation as part of a larger study entitled "Selection of Preservatives for Marine Structural Timbers in Herring Spawning Areas". In addition, these results were presented in poster format at the 2011 Alaska Marine Science Symposium and orally presented at the 2012 Alaska section of the American Fisheries Society meeting. Both studies will be submitted for publication in the journal Environmental Toxicology and Chemistry.

This research would not have been possible without expert training and guidance from many people. First and foremost were Mike Stekoll and Stanley "Jeep" Rice, both members of my committee and Mark Carls, Larry Holland, Marie Larsen, and Josie Lunasin at the Auke Bay Laboratory. Without their contribution of a wealth of knowledge, technical hydrocarbon training, and hydrocarbon analyses, this work would not have been possible. In addition, I thank Robert Perkins for the opportunity to participate in this project and for finding funding and providing engineering guidance and Anthony Gharrett for completing our committee. In addition, I'd like to thank these people for editing and contributing to this thesis. A special thanks to everyone who helped in the field, without whom, this work could not have been accomplished in a safe and efficient manner. A very special thanks to my friends and family, whose support and understanding kept me motivated throughout my graduate school experience.

GENERAL INTRODUCTION

Pacific herring (*Clupea pallasi*), valuable as both a commercial fish and as an important energetic prey species to many predators, has a vulnerable life stage when they can come into contact with toxic leachate from creosote infrastructure such as docks and harbors. The following introduction reviews herring ecology and creosote chemistry and the interaction between these complex subjects. Chapter One of this thesis will discuss toxicity measurements to embryos in controlled laboratory exposures; Chapter Two will discuss the environmental levels found in spawning habitat near creosote pilings.

Pacific herring distribution and value

Pacific herring are distributed widely throughout the coastal Pacific Ocean, from northern Baja California, north through Alaska, to Russia, Japan, the Yellow Sea, and adjacent seas (Lassuy 1989; Griffin et al.1998). These fish are an integral part of the marine ecosystem, both as secondary consumers and as forage fish for many species of birds, mammals, and fish. Herring have high energy content (2.17 kcal/g), and aggregate in large schools, making them important as a fishery, but probably more important as prey for a diverse array of predators they encounter at every life stage (Lassuy 1989). Pre-spawning aggregations are targeted by seabirds, humpback whales, and Steller sea lions. Their eggs are consumed by many shorebirds and invertebrates. As secondary consumers, Pacific herring feed on zooplankton in addition to large crustaceans and small fish. They provide an ecological link between secondary production and apex predators such as sea lions and whales in addition to piscivorous fishes such as salmon (Hay 1985; Norcross et al. 2001).

The Pacific herring fishery has a long history in Alaska, and continues to be economically valuable. They were first harvested by native peoples with nets, traps, and mazes (Lassuy 1989). In the 1900's continuing through the 1950's the fishery supported canned and salted herring markets in addition to fish meal and oil. The 1950's into the 1970's saw a decline in the demand for herring until Japanese import quotas were removed in the 1970's. This removal sparked a valuable roe fishery that continues to dominate herring harvests today (Lassuy

1989). The commercial Pacific herring fishery in Alaska is largely a sac and roe-on-kelp fishery for foreign markets, although small reduction, food, and bait fisheries exist. The 2011 total Pacific herring catch in Alaska was close to 45 million kg worth over 40 million dollars (Thynes et al. 2012). These harvests are largely accomplished using gillnets and purse seines (Lassuy 1989).

In southeast Alaska, Pacific herring support an important commercial fishery most notably in Sitka. Although there are five fishery stocks of Pacific herring in southeast Alaska (Sitka, Auke Bay, Craig-Hydaburg, Deer Island-Etolin Island, and Ketchikan) (Carlson 1980), the commercial harvest in recent years has been dominated by the Sitka stock, encompassing about 29,000 metric tons of the total 31,000 tons projected for harvest year 2012 (Thynes et al. 2012). The State of Alaska Department of Fish and Game (ADFG) manages the Pacific herring fishery using a limited entry system. Quotas are set to a maximum exploitation rate of 20% of estimated spawning biomass once the population has exceeded a threshold size for the local area. The Lynn Canal fishery near Juneau, Alaska, (the location of the current field study) has been closed since 1983. Managers cite overfishing, destruction of habitat (spawning grounds and eelgrass beds), dredging, and water quality degradation as possible causes for the decline (Thynes et al. 2012).

Pacific herring ecology

Pacific herring spawn at various times of the year depending on latitude. Spawn timing is highly variable but is closely related to sea surface temperatures in winter and spring (Norcross et al. 2001). Spawn timing exhibits a latitudinal gradient ranging from as early as October in California to as late as July in northern Alaska (Lassuy 1989). Spawn timing often coincides with local springtime conditions and increased plankton productivity. Spawning can occur in winter especially in southerly latitudes (Ware 1985; Lassuy 1989; Griffin et al. 1998; Norcross et al. 2001). Near Juneau, Alaska, Pacific herring typically spawn in April or May, but spawning may occur as late as June, corresponding to the spring zooplankton bloom (Carlson 1980; Quast 1986; Pritchett et al. 2007). Spawning, often occurring at night, takes place in a series of waves, each separated by days or weeks. This strategy aids survival by increasing the chances that some larvae will find plankton to feed on when their yolk sacs are depleted (Hay 1985; Lassuy 1989;

Norcross et al. 2001). Spring spawning coincides with warming sea temperatures which may cause increased leaching rates from creosoted pilings as solubilities of polycyclic aromatic hydrocarbons (PAHs) typically increase with increased temperature. The field portion of this study sought, in part, to determine whether creosote leach rates differ seasonally.

Pacific herring spawn in schools in the intertidal and shallow subtidal zones in sheltered inlets, sounds, bays, and estuaries (ADFG 2005). Spawning begins when males release milt and pheromones into the water stimulating females to release their eggs (Hay 1985; Lassuy 1989; Griffin et al. 1998, 2009). Each female deposits approximately 20,000 eggs within the subtidal and lower intertidal zones (Griffin et al. 1998). Newly spawned eggs are adhesive and readily attach to eelgrass, kelp, other marine vegetation, rocks, pier pilings, gravel, and each other (Alderdice and Hourston 1985; Haegele and Schweigert 1985; Lassuy 1989; Vines et al. 2000; ADFG 2005; O'Farrell and Larson 2005; Small et al. 2005; Griffin et al. 2009). Eggs may also be released directly into the water column (Hay 1985; Griffin et al. 2009). Less frequently, herring spawn along open coastlines (Hay 1985; Lassuy 1989; Vines et al. 2000). Herring show site fidelity, often returning to the same general locations to spawn year after year (Hay 1985; Lassuy 1989). Pacific herring may locate their spawning grounds by using hydrographic and topographic features (Hay 1985).

Pacific herring also spawn on man-made structures such as creosote pilings and rip-rap, especially when natural substrate is less abundant (Leet et al. 2001; Penttila 2007; Werme et al. 2010). Pacific herring eggs, once attached to substrate, are subject to environmental conditions until they hatch. Thus, eggs deposited on or near creosoted pilings are exposed to creosote and/or leachate throughout development. It has been shown that embryos developing on creosoted pilings exhibit high rates of mortality and deformities (Vines et al. 2000). Site fidelity could result in Pacific herring spawning on creosoted pilings year after year which may have population level consequences.

The duration of herring embryonic development and possible creosote exposure duration varies and is largely related to temperature, and in effect, latitude. In warmer temperatures and southerly latitudes herring embryos hatch after 8-10 days, whereas further north hatching can take as long as a month (Haegele and Schweigert 1985; Griffin et al. 2009). In Juneau, Alaska, embryonic development takes about 14-20 days depending on temperature (Quast 1986). Salinity also affects development duration. Too much deviation from population-specific

optimum salinity can result in longer development times and abnormalities, larval school separation, and inadequate energy reserves if yolk-sacs are prematurely exhausted due to late hatching. Longer development also increases the amount of time embryos are subject to predation, changing environmental conditions and exposure to water borne contaminants (Griffin et al. 1998).

Developing embryos of fishes are particularly sensitive to environmental toxicants because it is during this time that the major biological systems of organisms are being developed and larvae have high surface to volume ratios. Larval fishes are far less sensitive to toxicants than embryos, highlighting the sensitivity of the embryonic life stage (Rice and Harrison 1977). Timing of exposure during development is also important. Exposure during the first few cleavages of the fertilized egg often results in mortality, while teratogenic effects are more likely to occur during the pre-differentiated developmental stages and can occur at low level exposures (Hill and Johnston 1997; Hershberger et al. 2005). Biochemical and carcinogenic effects are associated with later development stages such as cellular and organ differentiation (Hershberger and Kocan 2000).

Natural mortality rates for Pacific herring embryos are generally high. The small, fertilized eggs adhere to vegetation and other substrates nearshore where they are vulnerable to predation, removal from substrate, overcrowding, and pollution. In the littoral zone the herring embryos are subject to variations in salinity, temperature, and dehydration (Haegele and Schweigert 1985; Lassuy1989; Ojaveer 2006). Too much deviation from optimal ranges, especially in temperature, can result in abnormalities and mortality (Lassuy 1989; Ojaveer 2006).

Creosote chemistry

Creosote is manufactured by high temperature distillation of coal tar,a byproduct ofthe coal carbonization process, and is commonly used in wood preservation to prevent degradation by marine borers and other organisms (Kang et al. 2005). Creosote can preserve wood for up to 100 years as compared to untreated wood that lasts about 10 years in the marine environment (Bestari et al. 1998a; Goyette and Brooks 1998; NOAA 2009). Even after 40 years of service, over half of the creosote will still be present in the wood (Bestari et al. 1998a). The toxicity and relative insolubility of creosote combined with its low cost make it the most effective and

popular wood preservation method (Kent 2007). For these reasons, creosote treated pilings are frequently used for building marine structures such as docks, harbors, and piers.

Creosote has been used to treat wood since 1948, before most environmental laws were in place. Despite its long history of use in the marine environment, robust scientific ecotoxicological data is lacking. Creosote is classified as a pesticide and is regulated under FIFRA (The Federal Insecticide, Fungicide, and Rodenticide Act). It was registered with the EPA (Environmental Protection Agency) in 1948 as part of the original enactment of FIFRA. It is classified as a restricted use pesticide that can only be applied with pressure treatment by certified entities (USEPA 2008; NOAA 2009).

The chemical composition of creosote is very complex and difficult to analyze (Lebow and Morrell 1988). In addition, the composition frequently varies depending on the origin of the coal and the distilling process employed. So far between 200 and 300 different components have been identified, many of which (45- 85%) are PAHs (Goyette and Brooks 1998; Vines et al. 2000; WHO 2004; USEPA 2008; NOAA 2009). There are two types of creosote: P2 that is used for railroad ties and P1/P13 used for aquatic applications and utility poles (USEPA 2008). According to WHO (2004), the American Wood Protection Association P1 type creosote contains 66% aromatic hydrocarbons, 0.82% tar acids/phenolics, 2.1% tar bases/nitrogen-containing heterocycles, 0.21% aromatic amines, 1.4% sulfur-containing heterocycles, 3.7% oxygen-containing heterocycles, and 23% other/not specified components. However, the concentration of each component group varies; tar acids and bases may contribute up to 5% each of the total composition of creosote (Kent 2007).

The PAHs in creosote contain between two and six fused aromatic rings. Both the parent compounds and their alkylated homologues are present. In general, the fingerprint of creosote is pyrogenic and different from crude oil in that the hydrocarbon pattern in creosote is dominated by parent compounds followed by a stair step decrease in the concentration of the alkylated homologues, whereas crude oil is dominated by alkylated homologues and the stair step pattern is reversed (Boehm et al. 1997; Neff 2002; Wang and Fingas 2003; USEPA 2008).

Creosote toxicity and bioavailability

The most toxic types of compounds in creosote are the phenols, cresols, and PAHs (Smith 2008). The PAHs are toxic to developing fish embryos at microgram per liter levels (Carls et al. 1999; Heintz et al. 1999; Barron et al. 2004; Incardona et al. 2004, 2008; Heintz 2007; Carls and Thedinga 2010; Hicken et al. 2011; Frantzen et al. 2012). For this reason, PAHs are the focus of creosote toxicity and weathering studies, including the current study. In general, lower molecular weight PAHs with two to three rings (naphthalenes, fluorenes, phenanthrenes, and anthracenes) are acutely toxic to aquatic organisms, while higher molecular weight PAHs with four to seven rings (pyrenes, chrysenes and benzo(a)pyrene) are not significantly acutely toxic but can be carcinogenic and toxic to embryos over a longer exposure period (Nagpal 1993; Carls et al. 1999; Heintz et al. 1999; Incardona et al. 2005). Creosote contains both types of PAHs, suggesting that it could have both acute and chronic toxic effects on fish.

The *Exxon Valdez* oil spill in 1989 generated a considerable amount of research on PAH toxicity to Pacific herring that can be applied to teleosts in general (Carls et al. 2008; Incardona et al. 2009). Polycyclic aromatic hydrocarbons such as naphthalenes, fluorenes, and phenanthrenes present in Alaska North Slope crude oil (ANSCO) are also present in marine grade creosote used to treat timbers, though the relative concentrations of compounds may be different (Barron et al. 2003; WHO 2004). The PAHs in oil and creosote most frequently associated with toxicity are fluorenes, dibenzothiophenes, and phenanthrenes (Incardona et al. 2009). Developing teleost embryos share common responses to PAH exposure and are sensitive to low-level PAH exposure, particularly during the first cleavage stages of embryogenesis (Hershberger et al. 2005; Carls et al. 2008; Incardona et al. 2009). Studies on the toxicity of ANSCO have shown that developing Pacific herring embryos are sensitive to aqueous PAH concentrations as low as 1-5 µg/L (Carls et al. 2008). Exposure in this range can result in yolk sac edema, while higher concentrations are associated with more obvious morphological defects, genetic damage, retarded growth, precocious hatching, reduction in swimming ability, and mortality (Carls et al. 1999, 2008; Hershberger et al. 2005; Hicken et al. 2011). Skeletal deformities and reduction in swimming ability resulting from embryonic PAH exposure in teleost fishes are well documented and have negative effects on long term survival. Other responses to PAH exposure observed in teleosts include premature or delayed hatching, shorter

length at hatch, hemorrhage, and yolk-sac asymmetry (Carls and Thedinga2010). The frequency of many of these responses are positively correlated with PAH concentration (Carls et al. 1999). The biological mechanisms that result in these responses in fish embryos may include cytotoxicity, DNA damage, and induction of cytochrome P4501A (Ownby et al. 2002).

The relative PAH concentrations of some components in oil can change and increase during the weathering process. The environmentally persistent, higher molecular weight PAHs, in addition to those with alkyl substitutions, can be detrimental to embryonic development at very low levels. For example, lowest observed effective concentrations (LOECs) are significantly lower for weathered oil (0.7-7.6 µg/L) than for less weathered oil (9-34 µg/L) (Carls et al. 1999, 2008; Short et al. 2003). These studies demonstrate that higher molecular weight, persistent PAHs are toxic to developing teleost embryos at microgram per liter levels. Due to creosote's longevity and slow weathering, it is possible that its weathered products, possibly present in the water column, may result in these same types of responses in Pacific herring and other marine species.

Exposure to sunlight in the water column increases the toxicity of some PAHs to microorganisms, fish, and plants. Enhanced toxicity due to light exposure (photosensitization or phototoxicity) is caused by activation of bioaccumulated PAH residues within the organism itself and leads to oxidation of membrane lipids, DNA, and proteins (Barron et al. 2003; Marwood et al. 2003). Sunlight increases the toxicity of PAHs to Pacific herring embryos 1.5-48 times and is aided in part by the lack of pigment and near transparency of embryos and larvae (Barron et al. 2003; NOAA 2009). Polycyclic aromatic hydrocarbons present in creosote and known to be phototoxic at low levels include anthracene, fluoranthene, pyrene, benzo(a)pyrene, and dibenzothiophene. In addition, PAH heterocycles such as acridine are also phototoxic (Barron et al. 2003).

Sixteen of the most ubiquitous compounds in creosote are on the EPA list of primary pollutants and are known to cause cancer, reproductive harm, and immune dysfunction, to impair normal development, and to have estrogenic qualities (Bestari et al. 1998a; NOAA2009). Both NOAA (National Oceanic and Atmospheric Administration) and the EPA have addressed creosote effects on fishes to varying degrees. The NOAA (2009) review of creosote effects on essential fish habitat concluded that PAH concentrations in water from creosote treated wood are unlikely to reach toxic levels in most cases due to water velocity and dilution. An exception may

be sensitive species such as Pacific herring that spawn on or near pilings as Vines et al. (2000) established. In addition, installation of new creosoted pilings in areas with elevated background PAH levels is discouraged. NOAA concluded that the main concern for creosote is PAH accumulation in the sediment which may negatively affect benthic habitat and productivity, especially for groundfish species. The use of creosote is banned in many freshwater areas on the west coast because of significant leaching (NOAA 2009).

Creosote toxicity has been studied in other species. For example, acute exposure of flat croaker (*Leiostomus xanthurus*), a coastal species, to creosote (320µg/L creosote-derived PAH) resulted in fin erosion and epidermal lesions. Embryonic exposure resulted in morphological abnormalities, yolk sac edema, anemia, reduced growth, and impaired swimming (NOAA 2009). A study conducted by Sibley et al. (2001) using marine-grade liquid creosote in freshwater microcosms found that creosote exposure negatively affected both zooplankton abundance and community structure. Acute toxicity was dose-dependent and the effective concentration resulting in a response in 50% of the population (EC50) for zooplankton for five and seven days post-treatment are 5.3 and 2.9 µg/L TPAH (total polycyclic aromatic hydrocarbons) respectively. After treatments, recovery of zooplankton communities was correlated with creosote concentration. Zooplankton are sensitive to creosote exposure resulting from a spill or localized leaching of creosote treated wood, and copepods appear to be especially sensitive (Sibley et al. 2001). In a similar experiment to Sibley et al. (2001), Marwood et al. (2003) determined that creosote inhibits photosynthesis and subsequent growth in Eurasian watermilfoil (*Myriophyllum spicatum*) and suggested that chronic low-level creosote exposure may have population level effects, although difficult to predict. Accumulation of PAHs associated with creosoted wood and contaminated sediment has been demonstrated in multiple aquatic species such as Oligochaete worms, Sydney rock oysters (*Saccostrea glomerate*), Pacific oysters (*Crasso streagigas*), and mussels (*Anodonta anatina*) (Hyötyläinen et al. 2002; Ownby et al. 2002; Smith 2008; Werme et al. 2010). Chronic, ultra-low level PAH concentrations (<1 µg/L PAHs) can accumulate in developing embryos and negatively affect fitness in later life stages and may have population level consequences (Short et al. 2003).

Individual creosote component EC50 reporting varies by organism, compound, and exposure duration. For this reason, it can be difficult to compare toxicities among organisms and compounds. For example, the 48 hour EC50 for anthracene in Mysid shrimp is 3.6 µg/L, while

for bluegill (*Lepomis macrochirus*) exposed for 96 hours, the EC50 is 1.27 µg/L. For sheepshead minnow (*Cyprinodon variegatus*), the 96 hour EC50 for fluoranthene is 0.8 µg/L, but 2200 µg/Lfor acenenaphthene (USEPA 2008). These EC50 values, while useful, illustrate the need to look at complex mixtures of chemicals in the environment because they rarely exist independently. The goal of this study is to further explore the toxicity of creosote to Pacific herring embryos and characterize environmental levels of creosote-associated mixtures.

Previous studies on creosote toxicity to Pacific herring embryos

The most recent and relevant study on the effects of creosote exposure to Pacific herring embryos is that of Vines et al. (2000). They conducted a series of experiments in 1995-1998 on the effects of creosote exposure on developing embryos and observed deleterious effects. In the first study, three sets of embryos were collected from a recent spawn at a marina approximately two days before hatching began: embryos attached to creosoted piling, embryos removed from a piling, and control embryos removed from a PVC pipe approximately 0.4 m away from a piling. Of the controls (removed from a PVC pipe), 96% hatched and 10% had morphological abnormalities. In contrast, 24% of the embryos removed from the piling hatched but none of the embryos that remained attached to creosote piling pieces successfully hatched. Morphological abnormalities, especially scoliosis, were observed in embryos exposed to creosoted pilings. In a second experiment, the LC50 (the concentration that is lethal to 50% of the study population), for hatching success of Pacific herring embryos for creosote was reported to be 50 µg/L diffusible-creosote using 10 different treatments (0-1500 µg/L) generated by incubating 1 g blocks cut from 40 year old creosote treated wood pilings in 16 ppt seawater for up to 24 hours. These studies indicate that diffusible-creosote has significant negative impacts to developing Pacific herring embryos, but whether dilution and tidal forces may provide protection for embryos developing unattached or a distance from creosoted pilings is unknown. Decreased hatching success and increased abnormal morphology was dependent on concentration and location from source. Embryos attached to pilings exhibited the most severe effects (Vines et al. 2000).

Environmental impact and fate of creosote

Structures built from creosote treated wood are long lasting, but the environmental impact and fate of components are not well understood. Migration of creosote from treated wood into the aquatic environment is affected by hydrodynamic forces, the retention level of creosote in the wood, water temperature, solubility, boiling points of components, and site-specific factors (Lebow and Morrell 1988; Bestari et al. 1998a; USEPA 2008). It has been shown that, typically, after new installations of creosote pilings, rapid leaching of PAHs and other components occurs, decreasing to a steady leaching rate within days to months. During this initial leaching period, localized contamination may occur. After the initial leaching period, PAH loss from creosoted pilings is thought to be slow and minimal. Salinity affects leaching rates, and leaching is greater in freshwater than in marine environments. Other variables that likely affect creosote leaching rates in the marine environment are current speed, water temperature, sun exposure, and biofouling. For these reasons, the toxicity risk to aquatic species post-installation is thought to be low and chronic in nature (Bestari et al. 1998a; Kang et al. 2005).

Polycyclic aromatic hydrocarbons are the main component of creosote and generally comprise the main focus of fate and risk assessments for creosote (USEPA 2008). In general, most PAHs are only slightly water soluble, nonionic, and once dissolved or suspended in water, are subject to removal processes: volatility, photodegradation, and microbial degradation (Bestari et al. 1998a; Vines et al. 2000; WHO 2004; Kang et al. 2005; USEPA2008). Typically for PAHs, solubility decreases with increasing molecular weight while resistance to oxidation, reduction, vaporization, and environmental persistence increases (Nagpal 1993; Goyette and Brooks 1998). Photo oxidation, whereby PAHs are degraded by light into different species, is an important environmental pathway. Photo oxidation of PAHs in surface water forms products that are more water soluble than the corresponding PAH. Photolytic products such as quinones, benzoic acids, and phenols are more soluble in water than the parent species and are persistent in air, water, and soils. Oxidized products are also more biologically available and potentially more acutely toxic than their parent compounds and can bioaccumulate (Marwood et al. 2003; USEPA 2008). PAHs migrating from creosoted pilings are likely influenced by all of these physical processes.

The relative composition of PAHs leaching from creosote treated wood into water post-installation varies temporally. Bestari et al. (1998b) observed the relative composition of 15 priority PAHs in water after adding different amounts of liquid creosote to freshwater microcosms. After two days, the relative composition in the water matched that of the liquid creosote and was dominated by two to five ringed PAHs. Through time, the concentrations of low and high molecular weight compounds decreased and the heaviest compounds were undetectable by 42 days. After 82 days, the composition by weight appeared Gaussian where it was dominated in concentration by intermediate weight compounds (four to five rings).

Creosote contaminated marine sediment may be a lasting point-source of PAHs that may have negative effects on biota. Sediment can be a repository but also point-source for PAHs migrating from creosoted pilings and is often the subject of creosote risk assessments (Sibley et al. 2001). Higher molecular weight compounds tend to adsorb onto organic matter particles and sediment where they can persist in the environment. The degree to which PAHs adsorb to and leach from sediment is affected by many of the same factors discussed above and is difficult to predict (Bestari et al. 1998b). Accumulation of PAHs originating from creosote in sediments in the marine environment has been documented. For example, Evans et al. (2009) found concentrations of PAHs exceeding probable effect levels for multiple compounds in sediments and impaired benthos communities near creosoted pilings at the Grey Owl Marina (built 1963) in Prince Albert National Park, Saskatchewan, Canada. The PAH concentration in sediments decreased substantially 2m away from pilings. They also noted that without adequate wave action and currents within protected harbors, PAHs are likely to linger near pilings in the water column. As part of a larger hydrocarbon study in Juneau, Alaska, sediments and caged mussels in marinas in Auke Bay and Aurora Harbor were documented to have concentrations of 4-5 and 2-6µg/g (dry weight) respectively of selected arenes in sediments. The authors concluded that these concentrations were likely to impair organisms (Ziemann and Fulton-Bennett 1990).

Objective

Pacific herring embryos were chosen for this study because they develop and hatch nearshore, sometimes spawning directly on creosoted structures. Spawning in the nearshore environment makes herring susceptible to exposure to PAHs and other compounds leaching from

creosoted pilings and other sources. Although creosote has been identified as toxic to Pacific herring embryos developing directly on creosoted pilings, it is unknown whether embryos developing nearby, such as on top of biofouling (mussels, barnacles, etc.), are also receiving toxic doses from the creosote. In addition, there is little information regarding the effects of sublethal doses of PAHs migrating from creosoted wood. The environmental footprint of PAHs leaching from creosoted structures in the subarctic marine environment is also largely unknown. The two studies in this thesis were conducted in order to better understand the toxicity of creosote to Pacific herring embryos and to explore real environmental PAH exposure scenarios near creosoted structures in a subarctic marine environment. These studies will provide much needed information regarding sublethal toxicity of creosote to Pacific herring embryos and whether embryos developing on biofouling or otherwise indirectly exposed are also at elevated toxicity risk. These studies combine laboratory generated toxicity data with environmental data collected at various marinas constructed from creosoted wood to improve our understanding of the toxicity risk of creosote to Pacific herring embryos.

References

ADFG (Alaska Department of Fish and Game). Divisions of Sport Fish and Commercial Fisheries. 2005. Special Publication No. 05-09 Commercial Fisheries of Alaska, Anchorage, AK. [cited 30 November 2013]. Available from: http://www.adfg.alaska.gov/FedAidPDFs/sp05-09.pdf. 9-17.

Alderdice DF, Hourston AS. 1985. Factors influencing development and survival of Pacific herring (*Clupea harengus pallasi*) eggs and larvae to beginning of exogenous feeding. *Can J Fish AquatSci*42:56-68.

Barron MG, Carls MG, Short JW, Rice SD. 2003. Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. *Environ Toxicol Chem* 22:650-660.

Barron MG, Carls MG, Heintz R, Rice SD. 2004. Evaluation of fish early life-stage toxicity models of chronic embryonic exposures to complex polycyclic aromatic hydrocarbon mixtures. *Toxicol Sci* 78: 60-67.

Bestari KT, Robinson RD, Solomon KR, Steele TS, Day KE, Sibley PK. 1998a. Distribution and composition of polycyclic aromatic hydrocarbons within experimental microcosms treated with creosote-impregnated Douglas fir pilings. *Environ Toxicol Chem*17: 2369–2377.

Bestari KT, Robinson RD, Solomon KR, Steele TS, Day KE, Sibley PK. 1998b. Distribution and composition of polycyclic aromatic hydrocarbons within experimental microcosms treated with liquid creosote. *Environ Toxicol Chem* 17: 2359-2368.

Boehm PD, Douglas GS, Burns WA, Mankiewicz PJ, Page DS, Bence AE. 1997. Application of petroleum hydrocarbon chemical fingerprinting and allocation techniques after the *Exxon Valdez* oil spill. *Mar Pollut Bull* 14:599-613.

Carls MG, Rice SD, Hose JE. 1999. Sensitivity of fish embryos to weathered crude oil: Part 1. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). *Environ Toxicol Chem* 18:481-493.

Carls MG, Holland L, Larsen M, Collier TK, Scholz NL, Incardona JP. 2008. Fish embryos are damaged by dissolved PAHs, not oil particles. *Aquat Toxicol* 88:121-127.

Carls M, Thedinga J. 2010. Exposure of pink salmon embryos to dissolved polynuclear aromatic hydrocarbons delays development, prolonging vulnerability to mechanical damage. *Mar Environ Res* 69:318-325.

Carlson HR. 1980. Seasonal distribution and environment of Pacific herring near Auke Bay, Lynn Canal, southeastern Alaska. *Trans Am Fish Soc* 109:71-78.

Evans MS, Fazakas K, Keating J. 2009. Creosote contamination in sediments of the Grey Owl Marina in Prince Albert National Park, Saskatchewan, Canada. *Water, Air, Soil Pollut* 20:161-184.

Frantzen M, Falk-Petersen IB, Nahrgang J, Smith T J, Olsen GH, Hangstad TA, Camus L. 2012. Toxicity of crude oil and pyrene to the embryos of beach spawning capelin (*Mallotus villosus*). *Aquat Toxicol 108:42-52.*

Goyette D, Brooks KM. 1998. Continuation of the Sooke Basin creosote evaluation study year four-Day 1360 & day 1540. Creosote Steering Committee Regional Program Report PR00-03.[cited 2 July 2014]. Available from: http://www.wwpinstitute.org/documents/SookeBasinRprt.pdf. 1-68.

Griffin FJ, Pillai MC, Vines CA, Kääriä J, Hibbard-Robbins T, Yanagimachi R, Cherr GN. 1998. Effects of salinity on sperm motility, fertilization, and development in the Pacific herring, *Clupea pallasi*. *Biol Bull* 194:25-35.

Griffin FJ, Smith EH, Vines CA, Cherr GN. 2009. Impacts of suspended sediments on fertilization, embryonic development, and early larval life stages of the Pacific herring, *Clupea pallasi*. *Biol Bull* 216:175-187.

Haegele CW, Schweigert JF. 1985. Distribution and characteristics of herring spawning grounds and description of spawning behavior. *Can J Fish Aquat Sci* 42 (Suppl. 1):39-55.

Hay DE. 1985. Reproductive biology of Pacific Herring (*Clupea harengus pallasi*). *Can J Fish Aquat Sci* 42 (Suppl. 1):111-126.

Heintz R, Short JW, Rice SD. 1999. Sensitivity of fish embryos to weathered crude oil: part II. Increased mortality of pink salmon (*Oncorhynchus gorbuscha*) embryos incubating downstream from weathered *Exxon Valdez* crude oil. *Environ Toxicol Chem* 18: 494-503.

Heintz RA. 2007. Chronic exposure to polynuclear aromatic hydrocarbons in natal habitats leads to decreased equilibrium size, growth, and stability of pink salmon populations. *Integr Enviro Assess Manage* 3:351-363.

Hershberger PK, Kocan RM. 2000. Egg incubation studies provide clues to the future of the once-largest herring stock in Puget Sound. *Puget Sound Notes* 44:5-9.

Hershberger PK, Elder NE, Wittouck J, Stick K, Kocan RM. 2005. Abnormalities in larvae from the once-largest Pacific herring population in Washington state result primarily from factors independent of spawning location. *Trans Am Fish Soc* 134:326-337.

Hicken CE, Linbo TL, Baldwin DH, Willis ML, Myers MS, Holland L, Larsen M, Stekoll MS, Rice SD, Collier TK, Scholz NL, Incardona JP. 2011. Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. *Proc Natl Acad Sci* 108:7086-7090.

Hill J. Johnston IA. 1997. Photomicrographic atlas of Atlantic herring embryonic development. *J Fish Biol* 51:960-977.

Hyötyläinen T, Karels A, Oikari A. 2002. Assessment of bioavailability and effects of chemicals due toremediation actions with caging mussels (*Anodonta anatine)* at a creosote-contaminated lake sediment site. *Water Res* 36: 4497-4504.

Incardona JP, Collier TK, Scholz NL. 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. *Toxicol Appl Pharmacol* 196:191-205.

Incardona JP, Carls MG, Teraoka H, Sloan CA, Collier TK, Scholz NL. 2005. Aryl hydrocarbon receptor–independent toxicity of weathered crude oil during fish development. *Environ Health Perspect* 113:1755-1762.

Incardona J, Carls MG, Day HL, Sloan CA, Bolton JL, Collier TK, Scholz NL. 2009. Cardiac arrhythmia is the primary response of embryonic Pacific herring (*Clupea pallasi*) exposed to crude oil during weathering. *Environ Sci Technol* 43: 201-207.

Kang SM, Morrell JJ, Simonsen J, Lebow S. 2005. Creosote movement from treated wood immersed in fresh water. *For Prod J* 55:42-46.

Kent JA. 2007. Kent and Riegel's Handbook of Industrial Chemistry and Biotechnology. Springer Science and Business Media LLC. New York.843-906.

Lassuy D.R. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest)--Pacific herring. U.S. Fish Wildl. Serv. Biol. Rep. 82(11.126). U.S. Army Corps of Engineers, TR-EL-82-4. . [cited 1 June 2014]. Available from: http://www.nwrc.usgs.gov/wdb/pub/species_profiles/82_11-126.pdf. 1-18.

Lebow ST, Morrell JJ. 1988. Exposure of creosoted Douglas-fir panels in Oregon coastal waters: a preliminary report. *For Prod J* 38:25-30.

Leet WS, Dewees CM, Klingbeil R, Larson EJ, editors. 2001. California's living marine resources: A status report. California Department of Fish and Game Resources Agency. 456-459. Martin G. 2009. Hard times for the bay all in a roe. *Bay Nature* January-March 38-43.

Marwood CA, Bestari KTJ, Gensemer RW, Solomon KR, Greenberg BM. 2003. Creosote toxicity to photosynthesis and plant growth in aquatic microcosms. *Environ Toxicol Chem* 22: 1075-1085.

Nagpal NK.1993. Ambient water quality criteria for polycyclic aromatic hydrocarbons (PAHs).Ministry of Environment, Lands and Parks Province of British Columbia. [cited 30 November 2013]. Available from: http://www.env.gov.bc.ca/wat/wq/BCguidelines/pahs/.

NOAA (National Oceanic and Atmospheric Administration). 2009. The use of treated wood products in aquatic environments: guidelines to west coast NOAA fisheries staff for the endangered species act and essential fish habitat consultations in the Alaska, Northwest and Southwest Regions. NOAA Fisheries-Southwest Region. [cited 12 January 2014]. Available from: http://www.westcoast.fisheries.noaa.gov/publications/habitat/treated_wood_guidelines_final_2010.pdf. 1-35.

Neff JM. 2002. Bioaccumulation in marine organisms: effect of contaminants from oil well produced water. Elsevier Inc., San Diego, California, USA. 241-314.

Norcross BL, Brown ED, Foy RJ, Frandsen M, Gay SM, Kline TC Jr., Mason DM, Patrick EV, Paul AJ, Stokesbury KDE. 2001. A synthesis of the life history and ecology of juvenile Pacific herring in Prince William Sound, Alaska. *Fish Oceanogr* 10: 42-57.

O'Farrell MR, Larson RJ. 2005. Year-class formation in Pacific herring (*Clupea pallasi*) estimated from spawning-date distributions of juveniles in San Francisco Bay, California. *Fish Bull* 103:130-141.

Ojaveer E. 2006.On the external and parental effects in early development of herring (*Clupea pallasi*) at the NE Kamchatka. *Fisheries Res* 81:1-8.

Ownby DR, Newman MC, Mulvey M, Vogelbein WK, Unger MA, Arzayus LF.2002. Fish (*Fundulus heteroclitus*) populations with different exposure histories differ in tolerance of creosote-contaminated sediments. *Environ Toxicol Chem* 21:1897-1902.

Penttila, D. 2007. Technical Report 2007-3 Marine forage fishes in Puget Sound. Washington Department of Fish and Wildlife in support of Puget Sound Nearshore Partnership, Olympia, Washington. [cited 2 July 2014]. Available from: http://www.pugetsoundnearshore.org/technical_papers/marine_fish.pdf 1-14. 1-20.

Pritchett M, Dressel S, Monagle K. 2007.Berners Bay Herring Research for 2005 and 2006Regional Information Report No. 1J07-01. Alaska Department of Fish and Game Division of Commercial Fisheries. Douglas, AK. 1-10.

Quast JC. 1986. Annual production of eviscerated body weight, fat, and gonads by Pacific herring, *Clupea harengus pallasi*, near Auke Bay, southeastern Alaska. *Fish Bull* 84:3 705-721.

Rice DW Jr.,Harrison FL. 1977. Copper sensitivity of Pacific herring, *Clupea harengus pallasi*, during its early life history. *Fish Bull* 76:347-356.

Sibley PK, Harris ML, Bestari KT, Steele TA, Robinson RD, Gensemer RW, Day KE, Solomon KR. 2001. Response of zooplankton communities to liquid creosote in freshwater microcosms. *Environ Toxicol Chem*. 20:394-405.

Short JW, Rice SD, Heintz RA, Carls MG, Moles A. 2003. Long-term effects of crude oil on developing fish: lessons from the *Exxon Valdez* oil spill. *Energy Sources* 25:509-517.

Small MP, Loxterman JL, FryeAE,VonBargen JF, Bowman C, Young SF. 2005. Temporal and spatial genetic structure among some Pacific herring populations in Puget Sound and the southern Strait of Georgia *Trans Am Fish Soc* 134:1329-1341.

Smith PT. 2008. Risks to human health and estuarine ecology posed by pulling out creosote-treated timber on oyster farms. *Aquat Toxicol* 86:287-298.

Thynes T, Gordon D, Monagle K, Walker S. 2012. 2012 Southeast Alaska Sac Roe Herring Fishery Management Plan. Alaska Department of Fish and Game Division of Commercial Fisheries. [cited 1 July 2014]. Available from: http://www.adfg.alaska.gov/FedAidpdfs/RIR.1J.2012.02.pdf. 1-25.

USEPA (U. S. Environmental Protection Agency). 2008. Reregistration eligibility decision for creosote (Case 0139). EPA 739-R-08-007 September 2008 (7510P). [cited 30 November 2013]. Available from:http://www.epa.gov/oppsrrd1/REDs/creosote_red.pdf. 1-40

Vines CA, Robbins T, Griffin FJ, Cherr GN. 2000. The effects of diffusible creosote-derived compounds on development in Pacific herring (*Clupea pallasi*). *Aquat Toxicol* 51:225-239.

Wang Z,Fingas MF. 2003. Development of oil hydrocarbon fingerprinting and identification techniques. *Mar Pollut Bull* 47:423-452.

Ware DM. 1985. Life history characteristics, reproductive value, and resilience of Pacific herring (*Clupea harengus pallasi*). *Can J Fish Aquat Sci* 42:127-137.

Werme C, Hunt J, Beller E, Cayce K, Klatt M, Melwani A, Polson E, Grossinger R. 2010. San Francisco Bay subtidal habitat goals report. Appendix 6-1: Removal of creosote treated pilings and structures from San Francisco Bay.[cited 30 November 2013]. Available from:http://www.sfbaysubtidal.org/PDFS/Ap6-1%20Creosote-Artificial%20Structures.pdf. 1-47.

WHO (World Health Organization). 2004. Concise International Chemical Assessment Document 62: Coal tar creosote. [cited 30 November 2013]. Available from: http://www.who.int/ipcs/publications/cicad/en/CICAD62.pdf. 1-90.

Ziemann DA, Fulton-Bennett KW. Editors. 1990. APPRISE- Interannual variability and fisheries recruitment. The Oceanic Institute. Honolulu, HI. 1-416.

CHAPTER 1: THE TOXICITY OF CREOSOTE TREATED WOOD TO PACIFIC HERRING (*CLUPEA PALLASI*) EMBRYOS[1]

Abstract

Pacific herring embryos are more sensitive to creosote exposure than previously documented. Creosote treated wood, a common building material for docks and harbors, is both a pesticide and a wood preservative composed of up to 85% polycyclic aromatic hydrocarbons (PAHs). In this study, Pacific herring embryos were exposed to water that had flowed past varying quantities of Best Management Practices creosoted wood in a flow-through dosing system]. Mean concentrations of total creosote-derived PAHs in exposures ranged from 0.12 to 30 µg/L. Exposure to creosote treated wood in low µg/L concentrations resulted in decreased hatch rates, increased incidence of skeletal defects, and impaired swimming ability. The presence of skeletal defects was the most sensitive and least variable indicator of creosote toxicity. The lowest observed effect concentration (LOEC) for skeletal defects was 6.8 µg/L (creosote derived total PAH) and the incidence of skeletal defects was often accompanied by ineffective swimming in exposed embryos. These responses have negative implications for survival and fitness; hatched larvae with skeletal defects are unable to forage and avoid predation and will likely not survive. These effects may have population level consequences.

[1] Duncan DL, Rice SD, Carls MG, Perkins R, Stekoll MS. Prepared or submission to Environmental Toxicology and Chemistry.

INTRODUCTION

Although creosote treated wood is commonly used to build marine structures such as docks and harbors, limited information exists regarding its toxicity to non-target organisms such as Pacific herring that spawn adhesive eggs nearshore on vegetation, rocks, and man-made structures including creosoted pilings (Haegele and Schweigert 1985; Vines et al. 2000; Leet et al. 2001; Penttila 2007; Werme et al. 2010). Pacific herring support commercial fisheries in Alaska, but are probably more important as widespread forage fish that support many parts of the ecosystem. Herring embryos developing on creosoted pilings exhibit high rates of mortality and deformities (Vines et al. 2000). However, it is unclear what effects creosoted wood may have on developing embryos that are not directly in contact with the pilings but are in close proximity, such as those embryos that are on fouling organisms that are attached to creosoted pilings. In this study, a flow-through dosing design using newly treated creosote treated wood (Best Management Practices (BMP)) was employed to determine the toxicity to Pacific herring embryos with exposures continuous throughout their embryonic development.

Creosote is used to prevent degradation by marine borers and other organisms and preserves wood for up to 100 years compared to untreated wood that lasts about 10 years in the marine environment (Bestari et al. 1998; Goyette and Brooks 1998; NOAA 2009). The chemical composition of creosote is complex; between 200 and 300 different components have been identified (Goyette and Brooks 1998; Vines et al. 2000; WHO 2004; USEPA 2008; NOAA 2009). Polycyclic aromatic hydrocarbons (PAHs) constitute a major component of creosote and are toxic to developing fish embryos at µg/L concentrations (Carls et al. 1999, 2008; Hershberger et al. 2005; Hicken et al. 2011). For this reason, PAHs are the focus of creosote toxicity and weathering studies including this study.

The *Exxon Valdez* oil spill in 1989 generated a considerable amount of research on PAH toxicity to Pacific herring that can be applied to teleosts in general (Carls et al. 2008; Incardona et al. 2009). Polycyclic aromatic hydrocarbons such as naphthalenes, fluorenes, and phenanthrenes present in Alaska North Slope crude oil(ANSCO) are also present in marine-grade creosote used to treat timbers, though the relative concentrations of compounds may be different (Barron et al. 2003; WHO 2004). Studies on the toxicity of ANSCO have shown that developing Pacific herring embryos are sensitive to aqueous PAH concentrations as low as 1-5 µg/L (Carls

et al. 2008). Exposure in this range can result in yolk sac edema while higher concentrations are associated with more obvious morphological defects, genetic damage, retarded growth, precocious hatching, reduction in swimming ability, and mortality (Carls et al. 1999, 2008; Hershberger et al. 2005). Chronic, low level PAH concentrations (<1 µg/L) can accumulate in developing embryos, negatively affecting fitness in later life stages, and may have population level consequences (Short et al. 2003).

The most recent and relevant study on the effects of creosote exposure to Pacific herring embryos is that of Vines et al. (2000). They conducted a series of experiments in 1995-1998 on the effects of creosote exposure on developing embryos observing deleterious effects. In one experiment, three sets of embryos were collected from a recent spawn at a marina approximately two days before hatching began: embryos attached to a creosoted piling, embryos removed from a piling, and control embryos removed from a PVC pipe approximately 0.4 m away from a piling. Of the control embryos 96% hatched and 10% had morphological abnormalities. In contrast, only 24% of the embryos removed from the piling hatched and none of the embryos attached to creosote piling pieces successfully hatched. Morphological abnormalities, especially scoliosis were observed in embryos exposed to creosoted pilings. In a second experiment the LC50 (the concentration that is lethal to 50% of the study population), for hatching success of Pacific herring embryos for creosote was estimated at 50 µg/L diffusible-creosote from 10 different treatments (0-1500 µg/L) of diffusible-creosote in seawater generated by incubating 1 g blocks cut from a 40 year old creosote treated wood piling in 16 ppt seawater for up to 24 hours.

The objective of the present study was to expand on the Vines et al. (2000) creosote toxicity study, by using a constant flowing water exposure system (that mimics the environmental exposure as much as possible), combined with characterization and quantification of the PAH exposure levels using GCMS instrumentation. It is important to be able to compare these results with previous herring PAH toxicity work done by both Vines et al. (2000) and *Exxon Valdez* researchers. In addition to quantifying the dosing, we also used several comparable end points: survival, hatching success, spinal deformities, and swimming performance. In this study, rather than using aged piling pieces, we exposed herring embryos to current BMP creosote treated wood, providing current, up to date toxicological information. These data will provide managers and engineers more information regarding the toxicity risk of creosote to developing Pacific herring embryos.

MATERIALS AND METHODS

Gravid Pacific herring were caught with jigs and beach seines during March-April of 2011 near Juneau, Alaska, in Seymour Canal (57.5°N latitude, 133.8°W longitude). Fish were kept alive in tanks at the NOAA Auke Bay Laboratory, until May 18, 2011 when they were used in this study. All experimental activities were conducted at the NOAA Auke Bay Laboratory Juneau, Alaska. Ovaries and testes were dissected and gametes harvested from 16 females and eight males by the method of Carls et al. (1999). The average gonadal somatic index (Hay 1985), a measure of gravidity, for females was 18 (SE=0.15) and for males was 14 (SE=0.37), indicating that the fish were in a "ripe" spawning state.

Neither eggs nor milt were pooled and each male fertilized the eggs of two females. From each of 16 ovaries, nine slides (25 X 75 mm) were prepared as described in Carls et al. (1999), for representation in each of nine treatments resulting in a total of 16 replicate slides per treatment. Approximately 100-200 eggs were attached to each slide by scooping eggs using a metal spatula from a longitudinally cut ovary, then swirling through the water such that eggs were spread evenly and in a single layer onto slides laid on the bottom of a clean, rectangular, Pyrex dish filled halfway with clean filtered seawater. The adhesive eggs readily attached to the slides and clean dishes were used for each ovary. Fertilization was achieved by placing the slideswith eggs attached and in slide racks, into a 1 L beaker filled with clean, filtered seawater. Within 30 minutes, a few milliliters of semen were added to the beaker. Using a clean stir bar and a stir plate, the gametes were mixed thoroughly for five minutes to ensure fertilization before moving into clean seawater. When all fertilizations were complete, the slide racks were moved into their respective treatments for the exposure period that lasted until the onset of hatching (approximately 14-15 days). At that time, the slides were removed from treatments and placed individually in 100 X 15 mm petri dishes and housed in a walk-in cooler at a temperature similar to Auke Bay seawater. Fresh seawater was provided every one to three days.

An additional five slides per ovary were prepared as above except that instead of fertilization in clean, filtered seawater, gametes were fertilized in their respective treatment solutions in order to test the effect of creosote exposure on fertilization. In addition, these slides were individually housed inside plastic bottles outfitted with Nitex screen to allow water flow and were not relocated at the onset of hatch. At 24 hours post-fertilization, excess embryos were

removed from the margins of slides with a razor blade where deposition was more than one layer deep. Due to the very small size of herring eggs (1.3-1.6 mm in diameter, Rice and Harrison (1977)) and lack of suitable imaging equipment, fertilization rates were not measured until experiment day 12 when photographs were taken and eyed and un-eyed embryos were counted. The mean eyeing rate on exposure day 12 was 82% which suggested sufficient fertilization success for the experiment.

To measure PAH uptake in embryos, additional eggs were applied to Nitex screen and fertilized as described. These were placed on the bottoms of treatment aquaria in both a high and a mid treatment, in addition to the water control. Subsamples of embryos on this screen were taken for hydrocarbon analysis on experiment days one, two, four, eight, and fifteen.

Plumbing and generator column contents

Embryos were exposed to creosote by continuously flowing fresh, filtered seawater past varying amounts of creosote treated boards nested within PVC pipe generator columns. Fresh, filtered seawater from Auke Bay was gravity-fed from a large Living Stream® aquaculture tank into a PVC manifold equipped with nine valves and outlets that fed nine separate PVC generator columns. For the controls and the four lowest treatments, 20 cm X 60 cm PVC generators were used. For the two highest treatments, larger 30 X 122 cm generators were used. Seawater flowed from the manifold into inlets at the base of the PVC columns, past the boards, and out the top through chemical-resistant Tygon tubing to the bottoms of 10 L polycarbonate aquaria. The aquaria housed the herring embryos which were attached to slides on slide racks or placed inside plastic bottles outfitted with Nitex screen. The aquaria were nested within a10 L Living Stream® tank supplied with running seawater that functioned as an insulating water bath. Flow rates were measured and adjusted daily to the target (500 ml/min) as needed. Seawater flowed through the system at a mean rate of 480ml/min (SE=3.4). The locations of the generator columns were randomized except for the two larger ones because of space constraints.

Wood description and preparation

On March 15, 2011, twenty 5 x 15 x 61 cm creosote treated boards were received from JH Baxter & Co through Oregon State University. They were recently treated using the current Best Management Practices according to the American Wood Preservers Association UC5 specifications for marine applications (personal communication J. Farley, J H. Baxter & Co). Using different amounts of creosoted boards to generate doses in a flow-through marine system was a novel approach. The goal was to attain seven treatments that had TPAH concentrations within the range of 1-100 µg/L, mimicking possible exposure scenarios in the environment. The weathering process was observed and concentration data were collected to determine amounts of wood needed for the exposure series. All of the creosoted wood used was weathered for 2-21 days prior to the experiment.

In order to achieve the targeted PAH concentrations in each treatment the following setup was used. There were a total of seven creosote treatments constructed with different amounts of treated wood. The two lowest treatments were created with a single, previously leached board cut into a 5 and a 15 cm piece respectively. The other creosote treatment generator columns contained one, two, four, and eight boards. For the two controls, one generator column was devoid of wood and the other contained one untreated Douglas fir board that was end-sealed.

Chemistry: environmental monitoring within treatment aquaria

Dissolved oxygen for all treatments was > 90% saturation measured using an YSI 55/12 FT portable dissolved oxygen meter on experiment days eight and thirteen. Nitrate and ammonia were below detection using an API aquarium test kit on experiment days 10 and 13. Temperature was measured daily and salinity was measured on experiment days 8, 10, and 13 with a YSI 30 Model 30/10 FT portable meter. Salinity for all treatments was 31 ppt. Temperature means during the experimental period for treatments in order of increasing mean TPAH were as follows: 6.4, 6.4, 6.5, 6.4, 7.1, and 7.5°C respectively. The water control, wood control, and water bath were 6.4, 6.8, and 6.6°C respectively. There was no significant difference in temperature between the lowest four treatments and the water control ($p \leq 0.05$). However, the two highest treatments and the wood control were significantly different from each

other and the lower four treatments in addition to the water control ($p \leq 0.05$). These treatments were generally warmer than the other treatments because they received more sunlight. This is a result of using larger PVC columns that were placed on the out-facing side of the exposure system.

Chemistry: aqueous TPAH

Aqueous TPAH concentration was measured as a surrogate for creosote concentration because of the chemical complexity of creosote and known toxicity of PAHs to fish embryos. Water samples (3.8 L) were taken from all treatments on exposure days 0, 1, 2, 4, 8, 12, 15, and 30 for TPAH analysis. A separate 4L glass jug was used to collect samples from each treatment. Samples were collected from effluent outlets and extracted within one hour with 75 ml methylene chloride twice after adding six deuterated PAH standards for recovery calculation. The extracts were stored at -20 °C until concentrating and exchanging for hexane on a steam bath. Samples were spiked with an internal instrument standard for concentration calculation and run in single ion monitoring mode on an Agilent 7890A gas chromatograph equipped with a model 5975C mass selective detector at NOAA Auke Bay Laboratories Ted Stevens Marine Research Institute according to the method of Short et al. (1996). Total PAH (TPAH) is reported as the sum of individual PAHs and their alkylated homologues ranging in size from naphthalenes (two rings) to benzo-g,h,i-perylene (six rings). Some samples were also analyzed in full scan mode to investigate the presence/absence of other components that may contribute to toxicity.

Embryo and larval measurements

The following responses were measured: fertilization success, hatching success, frequency of skeletal defect, and swimming performance. Fertilization success was measured on day 12 by counting the number of eyed and uneyed embryos using photographs obtained with a Canon EOS Rebel T2i camera with an ESF 18-55mm lens. Beginning at the onset of hatch (day 15), the remaining responses were observed and quantified on 25-75% of the slides daily. For each slide, the numbers of hatched larvae, live and dead, and the numbers of dead, eyed embryos

were recorded. The numbers of hatched larvae (live and dead) that had a skeletal defect were also tallied. A skeletal defect was noted if the spine had any kinks apparent to the naked eye. For live larvae, swimming performance was observed and classified as swimming normal, abnormal, or moribund. Swimming normal was defined as swimming in a typical "S" pattern, whereas swimming abnormal was swimming in any other way including sporadic twitching and swimming in circles. Larvae that were not actively swimming were given the opportunity to do so by gently sucking them up into a plastic pipette and releasing them back into the petri dish for a maximum of three times before classified as not swimming or moribund. Dead embryos and larvae were removed with a plastic pipette. All larvae alive at the time of observation were preserved in 10% neutral buffered formalin and placed in uniquely labeled glass vials after a lethal dose of MS-222 solution was administered in accordance with the University of Alaska Institutional Animal Care and Use Committee guidelines (see appendix for permit information).

PAH uptake

Embryos attached to Nitex screen were analyzed for hydrocarbons according to the method of Short et al. (1996). Briefly, embryo samples were extracted using a Thermo Scientific Dionex ASE system ™ whereby the organic compounds were extracted with methylene chloride under increased temperature and pressure. Then, samples were purified by both silica gel/alumina column chromatography and high performance liquid chromatography before analysis on a gas chromatograph/mass spectrometer as described above. Mean tissue sample weight was 1.7 g (SE=0.08). Six deuterated standards were added to samples pre-extraction to calculate PAH extraction recovery.

Data analysis

R Studio statistical software version 0.94.110 was used for all statistical analyses. Differences in temperature between treatments were estimated by analysis of variance (ANOVA) and Tukey contrasts. Differences in PAH compositions in treatments over time were investigated by analysis of covariance (ANCOVA). Where no interaction existed between experiment day and treatment (both statistically significant), the slopes, and thus leaching rates

of components were equal. Principal components analysis (PCA) was also used in the investigation of TPAH components across treatments.

The dose-response data collected were analyzed statistically and modeled by logistic regression. All responses were modeled as a function of the mean of the TPAH dose during the exposure period. The data sets showed some evidence of over-dispersion where there was more variability than expected from the logistic model. For this reason, the quasibinomial family was used instead of the binomial family and the resulting 95% confidence intervals are slightly larger to account for additional variability. The LC50, EC50, and EC20 values were estimated by logistic regression and the dose.p function in the MASS package of R Studio version 0.94.110 statistical software. The lowest test concentration that gives a mean response significantly different from that of the controls (LOEC) was determined using ANOVA and Tukey contrasts.

RESULTS

TPAH concentrations and composition

In general, creosote TPAH concentrations in the treatments decreased throughout the exposure period, with few exceptions (Fig. 1.1). Initial treatment concentrations were 3.1, 6.2, 5.8, 10, 14, and 33 µg/L TPAH. The means for the fourteen day exposure period were 1.8, 3.5, 4.3, 6.8, 16, and 30 µg/L TPAH and were used for statistical analyses. Final TPAH concentrations were 49 to 82% lower with a mean decrease of 69%. On the final exposure day, five of seven treatment concentrations were < 2 µg/L and the remaining two were 7 and 13 µg/L TPAH respectively. The mean TPAH concentrations for both the wood and water controls were ≤ 0.15 µg/L.

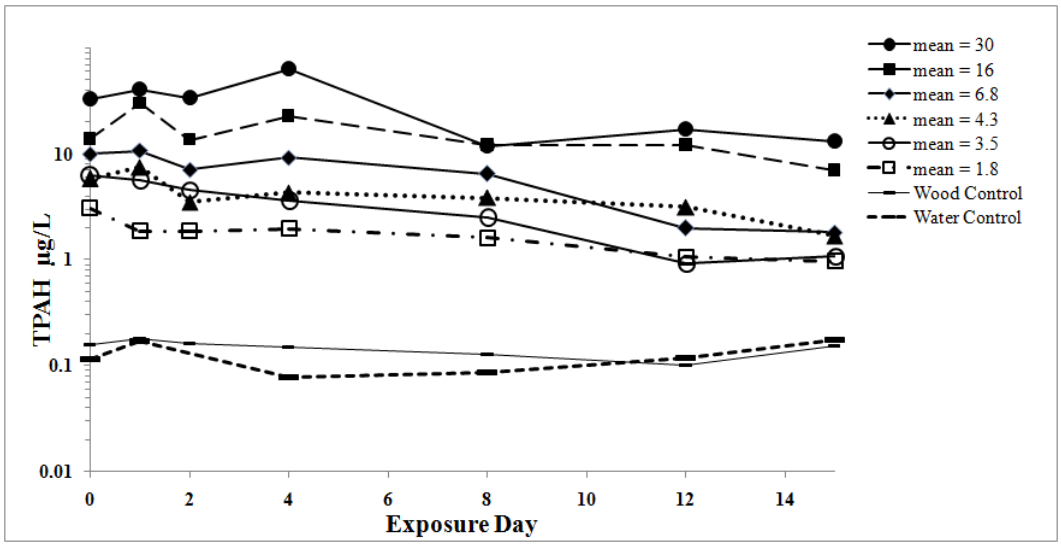

Fig.1.1.TPAH concentrations in creosote treatments. Note that the y-axis is a log scale.

Additionally the relative PAH composition of the treatments changed over time (Fig. 1.2). On experiment day one, treatment PAH compositions were dominated by 50% naphthalenes, followed by 13% each of acenaphthene and phenanthrenes, and 10% fluorene. In all cases, the proportion of the parent compound was greater than the homologues,consistent with creosote and pyrogenic composition. On experiment day 15, the proportion of naphthalenes and phenanthrenes decreased over 50%, while the proportion of acenaphthene nearly tripled. Despite the changes in relative proportion of these two and three ringed compounds, the treatments remained dominated by them, comprising 76 and 66% of the TPAH dose for days one and 15 respectively. All compounds heavier than pyrene comprised < 10% of the dose. However, it is worth noting that fluoranthene, with four rings, increased from < 1% of the relative composition on day one to 7% of the composition on day 15. Although the dose compositions were dominated by two and three ringed compounds, heavier, five ringed compounds such as benzo(a)pyrene and perylene were also present in the range of 1-10 µg/L. Full scan GCMS chromatograms of PAH extractions indicated that there was also a significant amount of phenols present in the treatments (phenols are toxic and highly water soluble (USHHS 2002). Leaching rates were tested by ANCOVA and no differences in slope as functions of treatment and time existed for naphthalene, phenanthrene, chrysene, or benzo(e)pyrene suggesting that leaching rates were approximately equal across treatments. PCA results detected

no differences in compositions among treatments; all treatments were distributed similarly and longitudinally in PCA space in the majority of observations.

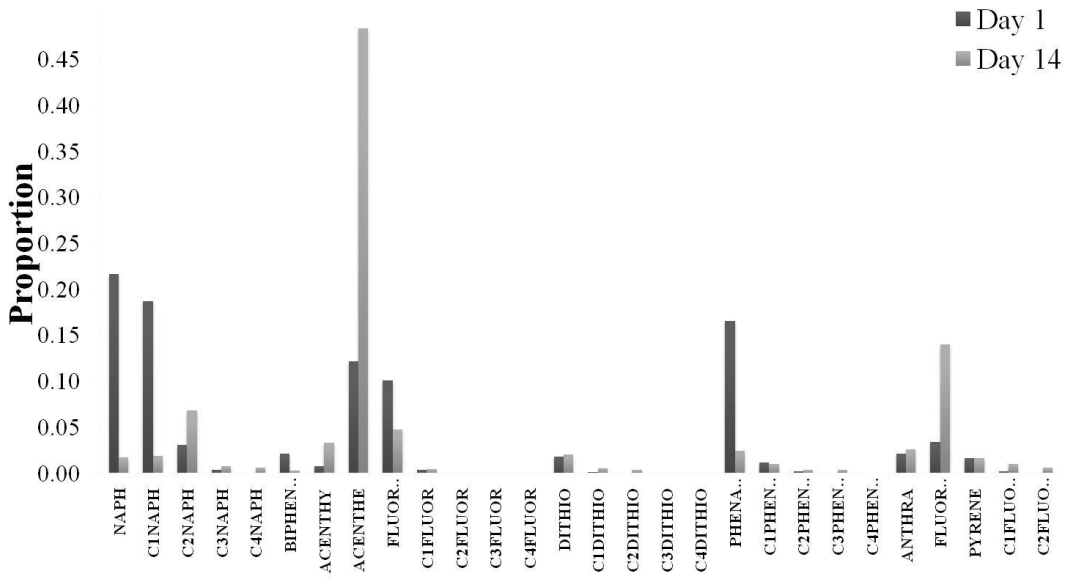

Fig.1.2. Change in creosote PAH composition as a function of time. PAHs larger than C2 flouranthenes had proportions < 1% of the total PAH and were omitted from the graph (shown is 16 µg/L treatment).

Uptake of PAH by embryos

The uptake of PAHs into embryo tissue was rapid; the highest concentrations were measured after one day of exposure to creosote treated wood (Fig. 1.3). Tissue concentrations declined over time similar to the exposure levels. Tissue TPAH concentrations in the exposed embryos were approximately 100 times greater than the exposure solution. The concentration in the embryos was well separated over time between the three treatment levels: control, 6.8 and 30 µg/L. Exposed embryos had significantly higher TPAH concentrations than the control group for both treatments ($p \leq 0.001$). Total PAH concentrations for embryos in treatment 6.8 µg/L differed significantly from those in treatment 30 µg/L ($p \leq 0.001$) (Fig. 2.3).

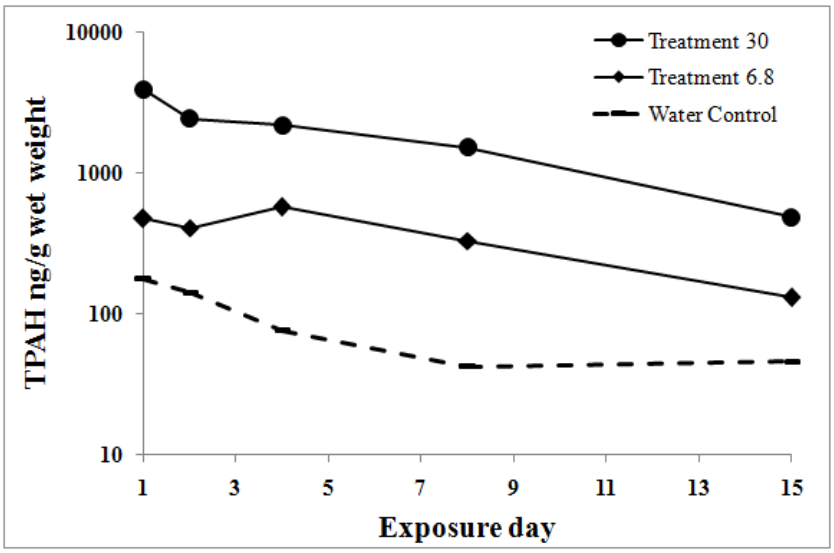

Fig. 1.3. PAH uptake in embryos. Note y axis is a log scale.

Biological responses to creosote exposure

Fertilization rates were not affected by exposure to creosote treated wood at the concentrations tested, as measured by survival to the eyeing stage at day 12. Mean eyeing rates for embryos fertilized in creosote treatment solutions and those fertilized in clean seawater on exposure day 12 were 84 and 81% respectively. Logistic regression of these data confirmed that eyeing rates were independent of TPAH concentration for embryos fertilized in treatment solutions ($p = 0.44$). This result is not surprising since the fertilization process is very rapid, and very early in the exposure test.

The LC50 for hatching success can only be estimated at between 5 and 50 µg/L creosote-derived TPAH because overcrowding led to high variance in the survival data (Fig. 1.4). Although the target loading was approximately 100 eggs per slide, in several cases, many more eggs were present and hatch rates suffered. To correct for overcrowding effects, only slides with 100 eggs or less were used for statistical analysis of hatching success, but this reduced the sample size from 144 to 37, and variance was significant. Despite overcrowding issues, creosote exposure was correlated with hatching success and hatch rates decreased with increasing dose ($p = 0.01$, Fig. 1.4).

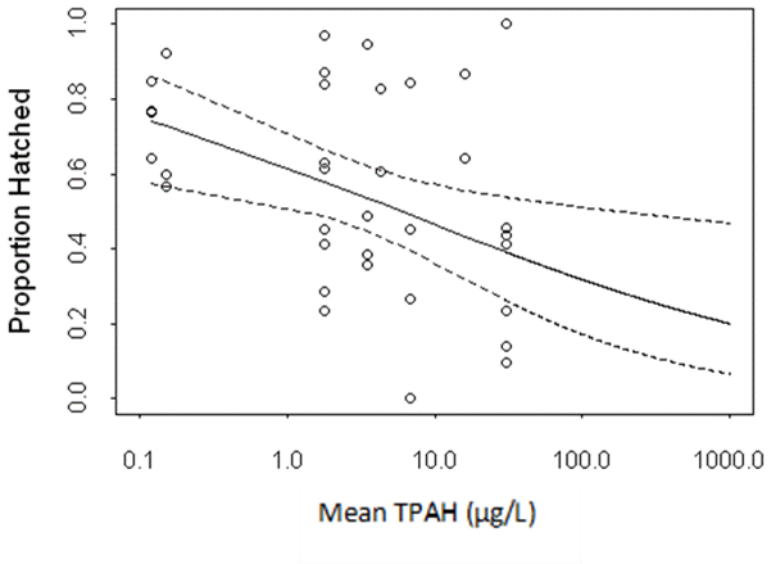

Fig. 1.4. Hatching success decreased with increasing creosote dose. Logistic regression line with 95% confidence bands (dashed lines).

The frequency of skeletal defects in hatched embryos and subsequent impaired swimming ability increased with dose ($p \leq 0.001$, Fig. 1.5). Significantly increased incidences of skeletal defects from that of the controls (the LOEC) occurred at 6.8 µg/L creosote-derived TPAH(SE = 0.43, $p \leq 0.001$). Skeletal defects were evident visually and often precluded swimming (Fig. 1.6). The EC20 and EC50 values were 9.4 (SE = 0.58) and 18 µg/L creosote-derived TPAH (SE = 0.80) respectively. The presence of skeletal defects and scoliosis in hatched larvae was the most sensitive and least variable effect of creosote exposure and was closely correlated with poor swimming ability. Impaired swimming included swimming in circles, half circles, and sporadic twitching. Larvae were also observed in a moribund state unable to swim. There was no correlation between hatching and skeletal defect rates indicating that skeletal observations were unaffected by culturing issues.

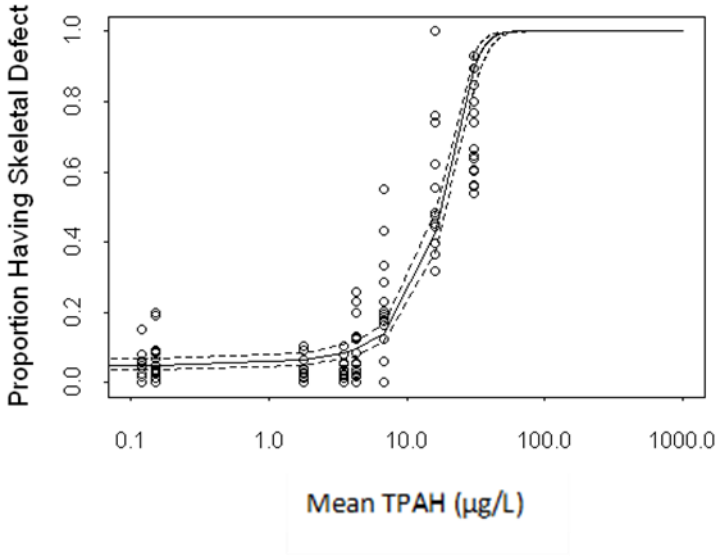

Fig.1.5. The frequency of skeletal defects increased with creosote dose. Logistic regression line with 95% confidence bands (dashed lines).

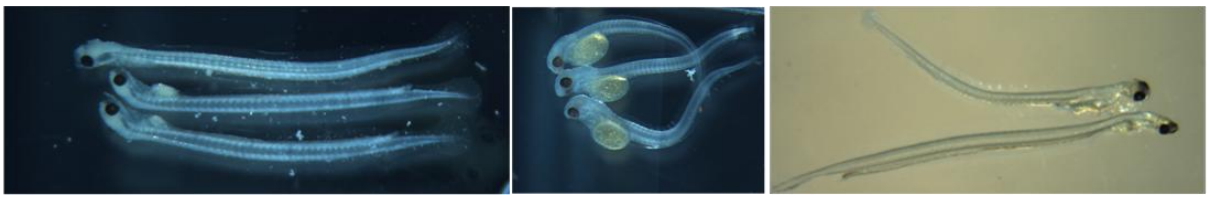

Fig.1.6. Pacific herring larvae from creosote treatments. From left to right: control group having no visible skeletal defect, 30 µg/L having skeletal defects/scoliosis, and side by side comparison of a larvae having a skeletal defect (top) and one without a defect (bottom). Note shorter length of deformed larvae.

DISCUSSION

Pacific herring embryos were sensitive to low microgram per liter concentrations of PAH from creosote exposures. The most sensitive and least variable indicator of creosote exposure was the incidence of skeletal defects and scoliosis in conjunction with decreased swimming performance. Both of these effects occurred at 6.8 µg/LTPAH and represent a LOEC for creosote not previously reported. Vines et al. (2000) reported an LC50 of 50 µg/L for survival at hatch when embryos were exposed to blocks cut from an existing creosoted piling at a harbor.

Our study reports an LC50 for hatching success that is somewhat lower (5-50 µg/L creosote-derived TPAH), but LC50s in general over-estimate a "safe" exposure level as compared to more sensitive effects, occurring at lower concentrations, such as scoliosis and other deformities. The LOEC dose of 6.8 µg/L is a more realistic estimate of a survival dose, because skeletal defects predict poor swimming performance, and poor survival.

Sometimes parameters such as swimming performance are reported as "sublethal", and their level of significance compared to "survival" can be difficult to interpret; but with larvae, any "sublethal" parameter that is observed is much more likely to mean death since larvae are extremely vulnerable. Injuries that affect their ability to feed or to avoid predation, even on the short term, will have direct impacts on their survival. For example, larvae with scoliosis, will not be able to feed or avoid predation, hence, these animals are effectively dead.

This study supports and builds upon the work of Vines et al. (2000) by reproducing a similar LC50 of near 50 µg/L, but lowers the LOEC to 6.8 µg/L for skeletal defects, thus lowering the estimated effective dose by nearly an order of magnitude. However, there are some notable differences between the studies. Our study used newly treated BMP creosote treated wood in a flowing marine environment, while the Vines study used blocks cut from an existing piling at a marina in a static environment. Skeletal defects and scoliosis were observed in both studies, but this study quantified these sublethal but detrimental effects. Vines et al. (2000) observed poor hatching success of larvae adhered to cut blocks of the creosoted piling, of which, none hatched, while 24% of larvae removed from the blocks hatched. These different exposure scenarios indicate that both newly treated BMP creosote and aged creosote are toxic to developing embryos at TPAH concentrations < 10 µg/L. These data sets are important to our understanding of creosote exposure effects on developing Pacific herring and other teleost embryos.

Creosote toxicity in herring embryos is not surprising, as there is a long history of documented PAH effects, particularly from oil spills. Teleosts share a common suite of responses to PAH exposure (Carls et al. 1999; Barron et al. 2003; Short et al. 2003; Incardona et al. 2009). The results of this experiment are similar to those previously published on the effects of PAH exposure in Pacific herring, pink salmon (*Oncorhynchus gorbuscha*), and zebrafish (*Danio rerio*). In weathered Alaska North Slope crude-oil experiments, concentrations ranging from 0.4-9.1 µg/L TPAH resulted in a multitude of responses including morphological defects,

increased mortality, yolk-sac edema, and impaired swimming in embryos exposed during development. The sublethal responses observed in this study: skeletal defects, scoliosis, and reduced swimming performance, reduce survival in Pacific herring and pink salmon (Carls et al. 1999, 2008).

Cardiac defects, the most sensitive indicator of PAH exposure in teleosts are well documented and include edema, changes in heart shape and rhythm, and heart failure (Hicken et al. 2011; Incardona et al. 2009; Dubansky et al. 2013). Sublethal oil exposures in developing zebrafish embryos have shown that a 9% decrease in length-to-width ratio in the heart results in an 18% reduction in U_{crit} (a measurement of sustained swimming ability) as adult fish (Hicken et al. 2011). These reductions in swimming performance negatively affect fitness and survivorship by reducing the ability to maintain position in the water column and effectively forage and avoid predation (Hicken et al. 2011). Other morphological deformities observed in fishes associated with PAH exposure are yolk sac edema, small jaws, size reduction, and body axis defects (Incardona et al. 2004, 2009; Dubansky et al. 2013).

PAH exposure to fishes can have population level consequences. Decreased hatch rates can result in lower recruitment for young of the year. In addition, larvae with reduced swimming ability are less able to capture prey and avoid predation (Carls et al. 1999). For example, after the Prince William Sound oil spill in 1989, there was a 52% reduction of Pacific herring larval survival at and near oiled beaches (Carls et al. 1999). Many of these fish exhibited signs of PAH exposure such as skeletal and cranio-facial deformations, genetic damage, and decreased body size (Carls et al. 1999). In a separate study, oceanic survival was reduced for pink salmon embryos exposed to low level, chronic concentrations of weathered oil despite having no visible defects upon release into the environment (Heintz et al. 2000). More recently, similar effects have been observed following the Deep Water Horizon oil spill. Gulf killifish (*Fundulus grandis*) exposed to oiled marsh sediment exhibited delayed and reduced hatching success (Dubansky et al. 2013). These population level consequences may have unforeseeable effects on the ecosystem as a whole. In addition, fishery revenues may be lost when populations are negatively affected by oil spills and other PAH sources. Revenues may be lost in the form of fishery closures and/or population declines or failure to recover.

Creosote treated wood in the aquatic environment is a long-lived point source of PAHs and may have negative effects on habitat and fish populations. The PAHs migrate out of

impregnated wood and into the surrounding water where they undergo weathering processes previously described and may adsorb onto sediment and persist for an unknown amount of time (Evans et al. 2009). In addition, some of the compounds found in creosote such as anthracene, fluoranthene, pyrene, and benzo(a)pyrene are phototoxic, presenting an additional exposure pathway for translucent herring embryos and larvae such as Pacific herring developing in the nearshore environment (Barron et al. 2003).

Creosote treated wood in the aquatic environment can be toxic to herring embryos if the PAH leachate reaches the low microgram per liter level; our most sensitive response was deformities, which occurred at an exposure level of 6.8 µg/L TPAH following exposure of embryos to creosote treated wood. This toxicity is not surprising, since PAHs constitute large percentages of the composite mixture in creosote, and treated wood has a long life expectancy with the ability to leach over a long period of time. Previous work, primarily with oil spills, has demonstrated that PAHs affect fish embryos at low microgram per liter levels.

CONCLUSION

Our toxicity results indicate that Pacific herring embryos are more sensitive to creosote treated wood than previously documented; skeletal deformities occurred at 6.8 µg/L TPAH. Skeletal deformities, likely to result in mortality, were the most important and sensitive response. Uptake of PAHs into embryos was very rapid, but fertilization success was unaffected by creosote leachate exposure. Embryos attached to creosoted pilings may have even higher mortality rates (direct contact would facilitate more efficient tissue uptake of toxic concentrations of creosote components such as PAHs). However, there likely is rapid dilution in the environment, and a field study would be able to determine if toxic environmental levels of creosote components can be achieved at various distances from pilings in the nearshore environment.

Acknowledgements

We thank The Alaska Department of Transportation and the Institute for Northern Engineering at the University of Alaska Fairbanks for funding this project and the NOAA Auke Bay Laboratory for supplying expert guidance, fish, laboratory space, and chemical analyses. A special thanks to Marie Larsen and Josie Lunasin who provided many hours of training and analyses without which, this project could not have been completed. We also give thanks to Jeff Morrell at Oregon State University for providing the creosote treated wood and the University of Alaska Institutional Animal Care and Use Committee for animal handling guidance (permit #210243-3).

References

Barron MG, Carls MG, Short JW, Rice SD. 2003. Photo enhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to Pacific herring eggs and larvae. *Environ Toxicol Chem* 22:650-660.

Bestari KTJ, Robinson RD, Solomon KR, Steele TS, Day KE, Sibley PK. 1998. Distribution and composition of polycyclic aromatic hydrocarbons within experimental microcosms treated with creosote-impregnated Douglas fir pilings. *Environ Toxicol Chem* 17: 2369–2377.

Carls MG, Rice SD, Hose JE. 1999. Sensitivity of fish embryos to weathered crude oil: Part 1. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). *Environ Toxicol Chem* 18:481-493.

Carls MG, Holland L, Larsen M, Collier TK, Scholz NL, Incardona JP. 2008. Fish embryos are damaged by dissolved PAHs, not oil particles. *Aquat Toxicol* 88:121-127.

Dubansky B, Whitehead A, Miller JT, Rice CD, Galvez F. 2013. Multitissue molecular, genomic, and developmental effects of the *Deepwater Horizon* oil spill on resident gulf killifish (*Fundulus grandis*). *Environ Sci Technol* 47:5074-5082.

Evans MS, Fazakas K, Keating J. 2009. Creosote contamination in sediments of the Grey Owl Marina in Prince Albert National Park, Saskatchewan, Canada. *Water, Air, Soil Pollut* 201:161-184.

Goyette D, Brooks KM. 1998. Continuation of the Sooke Basin creosote evaluation study year four-Day 1360 & day 1540. Creosote Steering Committee Regional Program Report PR00-03. [cited2July 2014]. Available from: http://www.wwpinstitute.org/documents/SookeBasinRprt.pdf. 1-68.

Haegele CW, Schweigert JF. 1985. Distribution and characteristics of herring spawning grounds and description of spawning behavior. *Can J Fish Aquat Sci* 42 (Suppl. 1):39-55.

Hay, DE. 1985. Reproductive Biology of Pacific Herring (*Clupea harengus pallasi*). *Can J Fish Aquat Sci* 42:111-126.

Hershberger PK, Elder NE, Wittouck J, Stick K, Kocan RM. 2005. Abnormalities in larvae from the once-largest Pacific herring population in Washington state result primarily from factors independent of spawning location. *Trans Am Fish Soc* 134:326-337.

Heintz R, Rice SD, Wertheimer AC, Bradshaw RF, Thrower FP, Joyce JE, Short JW. 2000. Delayed effects on growth and marine survival of pink salmon *Oncorhynchus gorbuscha* after exposure to crude oil during embryonic development. *Mar Ecol Prog Ser* 208:205-216.

Hicken CE, Linbo TL, Baldwin DH, Willis ML, Myers MS, Holland L, Larsen M, Stekoll MS, Rice SD, Collier TK, Scholz NL, Incardona JP. 2011. Sublethal exposure to crude oil during embryonic development alters cardiac morphology and reduces aerobic capacity in adult fish. *Proc Natl Acad Sci* 108:7086-7090.

Incardona JP, Collier TK, Scholz NL, 2004. Defects in cardiac function precede morphological abnormalities in fish embryos exposed to polycyclic aromatic hydrocarbons. *Toxicol and Appl Pharmacol* 196:191-205.

Incardona J, Carls MG, Day HL, Sloan CA, Bolton JL, Collier TK,Scholz NL. 2009. Cardiac arrhythmia is the primary response of embryonic Pacific herring (*Clupea pallasi*) exposed to crude oil during weathering. *Environ Sci Technol* 43: 201-207.

Leet WS, Dewees CM, Klingbeil R, Larson EJ, editors. 2001. California's living marine resources: A status report. California Department of Fish and Game Resources Agency. 456-459.

NOAA (National Oceanic and Atmospheric Administration). 2009. The use of treated wood products in aquatic environments: guidelines to west coast NOAA fisheries staff for the endangered species act and essential fish habitat consultations in the Alaska, Northwest and Southwest Regions. NOAA Fisheries-Southwest Region. [cited 12 January 2014]. Available from:
http://www.westcoast.fisheries.noaa.gov/publications/habitat/treated_wood_guidelines_final_2010.pdf. 1-35.

Penttila, D. 2007. Technical Report 2007-3 Marine forage fishes in Puget Sound. Washington Department of Fish and Wildlife in support of Puget Sound Nearshore Partnership, Olympia, Washington. [cited 2 July 2014]. Available from:
http://www.pugetsoundnearshore.org/technical_papers/marine_fish.pdf 1-14. 1-20.

Rice DW Jr., Harrison FL. 1977. Copper sensitivity of Pacific herring, *Clupea harengus pallasi*, during its early life history. *Fish Bull* 76:347-356.

Short JW, Jackson TJ, Larsen ML, Wade TL. 1996. Analytical methods used for the analysis of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resources damage assessment of the *Exxon Valdez* oil spill. *Am Fish Soc Symp* 18:140–148.

Short JW, Rice SD, Heintz RA, Carls MG, Moles A. 2003. Long-term effects of crude oil on developing fish: lessons from the *Exxon Valdez* oil spill. *Energy Sources* 25:509-517.

USEPA (U. S. Environmental Protection Agency). 2008. Reregistration eligibility decision for creosote (Case 0139). EPA 739-R-08-007 September 2008 (7510P). [cited 30 November 2013]. Available from: http://www.epa.gov/oppsrrd1/REDs/creosote_red.pdf. 1-40

USHHS (United States Department of Health and Human Services). 2002. Toxicological profile for wood creosote, coal tar creosote, coal tar, coal tar pitch, and coal tar pitch volatiles. Atlanta, Georgia. [cited 2 July 2014]. Available from:http://www.atsdr.cdc.gov/toxprofiles/tp85-p.pdf. 1-248.

Vines CA, Robbins T, Griffin FJ, Cherr GN. 2000. The effects of diffusible creosote-derived compounds on development in Pacific herring (*Clupea pallasi*). *Aquat Toxicol* 51:225-239.

Werme C, Hunt J, Beller E, Cayce K, Klatt M, Melwani A, Polson E, Grossinger R. 2010. San Francisco Bay subtidal habitat goals report. Appendix 6-1: Removal of creosote treated pilings and structures from San Francisco Bay.[cited 30 November 2013]. Available from:http://www.sfbaysubtidal.org/PDFS/Ap6-1%20Creosote-Artificial%20Structures.pdf. 1-47.

WHO (World Health Organization). 2004. Concise International Chemical Assessment Document 62: Coal tar creosote. [cited 2 July 2014]. Available from: http://www.who.int/ipcs/publications/cicad/en/CICAD62.pdf. 1-90.

CHAPTER 2: CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS NEAR CREOSOTED PILINGS IN JUNEAU, ALASKA[2]

Abstract

Total polycyclic aromatic hydrocarbons (PAHs) were measured with passive sampling devices at three distances (10 cm, 1 m, and 10 m) from creosoted pilings at three docks in Juneau, Alaska. In addition, PAHs were measured on the surface of a piling at one harbor. Total PAHs were significantly elevated during late summer and correlated with temperature. Boating activity may also have contributed to seasonal increases. Elevated hydrocarbon concentrations closest to creosoted pilings were observed at Aurora Harbor where PAH concentrations 10 cm and 1 m from pilings were significantly higher than near the harbor opening. This trend was not observed at the Indian Point Field Office or the Auke Bay Marine Station where aqueous PAH concentrations were significantly higher. In some cases, primarily within 10 cm from creosoted pilings, concentrations exceeded the laboratory established lowest observed effect concentration (6.8 µg/L total polycyclic aromatic hydrocarbons equal to 81,000 ng/g passive sampling device) for skeletal defects in Pacific herring embryos. The PAH compositions of the field samples were largely pyrogenic and some were consistent in composition with creosote. There is previous evidence that Pacific herring embryos developing directly on creosoted pilings exhibit increased mortality rates. Results reported here suggest that in some cases, there may be a zone of increased polycyclic aromatic hydrocarbon loading near creosoted pilings, indicating that embryos do not have to be attached to pilings to suffer adverse effects. In addition, seasonal changes in temperature and boating activity likely contribute to the overall aqueous hydrocarbon concentration at docks and harbors.

[2] Duncan DL, Rice SD, Carls MG, Stekoll MS. Prepared for submission to Environmental Toxicology and Chemistry.

INTRODUCTION

Creosote has been reported to be toxic to Pacific herring embryos in two independent laboratory studies (Vines et al. 2000; Duncan 2014). Pacific herring and developing embryos may come in contact with creosote treated wood because they spawn in schools in the intertidal and shallow subtidal zones in sheltered inlets, sounds, bays, and estuaries; newly spawned eggs are adhesive and readily attach to eelgrass, kelp, other marine vegetation, rocks, pier pilings, gravel, and each other (Alderdice and Hourston 1985; Haegele and Schweigert 1985; Lassuy 1989; Vines et al. 2000; ADFG 2005; O'Farrell and Larson 2005; Small et al. 2005; Incardona et al. 2008; Griffin et al. 2009). However, whether environmental PAH concentrations near creosoted structures are sufficient to have toxic effects on Pacific herring embryos is unknown. It is unclear whether embryos developing on top of fouling organisms (a few centimeters from the piling) are also at elevated toxicity risk, or if dilution, water currents, and tidal forces preclude toxic aqueous concentrations. In addition, little is known about the environmental footprint and leaching behavior of creosote treated wood in the sub-arctic marine environment.

To answer these questions, this study was conducted to quantify and characterize both environmental PAH concentrations and their compositions near creosote treated wood structures in Juneau, Alaska, and compare these results with a previous laboratory toxicity study (Duncan 2014). We determined the potential exposure of developing herring to creosote in the water by placing passive water sampling devices at varying distances from creosote treated wood structures at three docks in the Juneau area. Relative PAH concentrations were determined by GCMS analysis, which also determined the composition of hydrocarbons to help in source identification. These data were compared to distance from creosoted pilings, site sampled, and dates deployed (to determine if a suspected seasonal activity change would have an impact). These data were compared with similar laboratory and historic data to explore the exposure risk to developing Pacific herring embryos near creosoted pilings.

Field sampling of low-level contaminants is challenging; direct sampling will give accurate results if the contaminant is above detection levels, but sampling a “snap shot” in time may easily miss intermittent contamination events. Passive sampling captures intermittent events and is very sensitive, but the sampling rate (the amount of water sampled) and contaminant quantification are confounded by natural processes and factors such as temperature,

tides, and current speed, in addition to exposure duration (Carls et al. 2008; Bao et al. 2012). For example, it may be impossible to determine the timing or frequency at which a particular contaminant enters the water, and what the incoming concentration was, making it difficult to back-calculate what the actual water concentrations were at any moment in time. Despite these caveats, passive sampling devices were used in this environmental monitoring study because they are extremely sensitive, especially for minute and/or intermittent contaminants and can aid in the identification of contaminated versus uncontaminated sites (Carls et al. 2004, 2008; Adams et al. 2007; Bao et al. 2012). Passive sampling captures the "total exposure", even if it is intermittent. These devices measure the dissolved and bioavailable fraction of contaminants and have been employed in toxicity, weathering, and risk assessment studies (Anderson et al. 2008, Allan et al. 2011). Passive sampling devices have been field and laboratory verified to provide accurate, time-integrated hydrocarbon data from environmental water samples (Carls et al. 2004; Allan et al. 2010).

MATERIALS AND METHODS

Passive samplers

Passive samplers were manufactured, deployed, retrieved, extracted, and analyzed for PAHs by using the method of Carls et al. (2004). Passive samplers were constructed from low-density polyethylene tubing without additives (thickness, ~98 µm, Brentwood Plastics, St. Louis, MO, USA). In the literature, passive samplers are sometimes referred to as LDPE (low-density polyethylene) or PEMDs (polyethylene membrane devices). Segments of tubing of two lengths (50 and 70 cm) were cut and split to form 4.9 cm wide strips. These strips were hydrocarbon cleaned by sonicating in pentane for 15 minutes, then soaking for three, 30 minute intervals in pentane. The strips were loaded into either pucks (cylindrical stainless steel housings having perforated stainless steel tops and bottoms) or attached to stainless steel halibut clips, depending on the application. Both pucks and clips were cleaned prior to use by soaking and agitating in methylene chloride, followed by drying. Clips were used for samples located sufficiently far from creosoted pilings so as to not have direct contact with the pilings. Pucks were used for all 10 cm samples ensuring that the passive samplers were not in direct contact with creosoted

pilings. Pucks allowed water to freely flow through the sampler while keeping the passive sampler secure. After the pucks were loaded with the sampling strips, they were double-wrapped in aluminum foil and double heat sealed in Ziploc® freezer bags. A lab sample was taken from each batch of 15 prepared pucks and analyzed to verify pre-deployment cleanliness. Retrieved pucks and clips were also double-wrapped in aluminum foil and double-Ziploc bagged. To ensure field cleanliness, an unopened trip blank was analyzed in addition to a field blank that was opened and exposed to the air for approximately two minutes. Upon retrieval, strips were unloaded from pucks and clips with hydrocarbon clean tools. If not immediately unloaded, pucks and clips were stored in a freezer set to -20 °C.

Extraction and chemical analyses

PAH extraction consisted of first adding six deuterated PAH recovery standards in hexane solution to the passive sampler strips. The strips were placed in hydrocarbon-clean glass vessels covered in aluminum foil and sonicated in 80:20 pentane: methylene chloride for 20 min. followed by a 30 min. soak period in triplicate. Upon the final sonication, strip extracts were dehydrated over sodium sulfate, concentrated on a steam bath, and exchanged for hexane. Concentrated samples were passed through a 1.5 g silica gel column to remove extraneous compounds. The extracts were spiked with an instrument standard before running on an Agilent 7890A gas chromatograph equipped with a model 5975C mass selective detector at the NOAA Auke Bay Laboratories Ted Stevens Marine Research Institute according to the method of Short et al. (1996).

Field sites

In order to characterize environmental hydrocarbon exposures in the field, passive samplers were deployed at two docks and one harbor in Juneau, Alaska, during the fall of 2011 and the summer of 2012. The first sampling period was a single deployment in October of 2011. The second sampling was completed over the summer of 2012 and involved both continuous and monthly deployments. The same field sites were sampled during both parts of the study. Each

sampling period lasted for 14 days in order to compare data with those of a previous laboratory toxicity experiment (Duncan 2014).

The sites were chosen because they were easily accessed, spatially and functionally different, and represent three different creosote exposure scenarios. The sites were as follows: Aurora Harbor (AH), 1423 Harbor Way, the dock at the National Marine Fisheries Service, Auke Bay Marine Station (ABMS), 11305 Glacier Hwy, and the National Park Service Indian Point Field Office (IPFO) dock at 3100 National Park Rd (Fig. 2.1). All three locations were built in the early to mid 1960's (personal communications: Bob Barte (NPS), Stanley Rice (NOAA NMFS), and Dwight Tajon (Juneau harbormaster)). AH is the sole harbor in the study and is the largest public harbor in Juneau consisting of approximately 260 creosoted pilings in an area approximately 0.1 km^2 and partially enclosed by riprap and breakwater. The harbor has the capacity to moor 309 24-32 ft vessels, 66 vessels larger than 42 ft, and has covered moorage for an additional 42 vessels. Transient moorage is not allowed in AH and is home to many people living on private, domestic vessels as well as recreational and commercial fishing vessels (CBJ 2013). The ABMS dock is in Auke Bay, an area encompassing approximately 11.5 km^2 (McGurk 1989). ABMS is located on the northeast side of the bay within a relatively developed marine area. Nearby are a series of docks and harbors: Statter harbor, Andrews Marina, and Fisherman's Bend. The Alaska State Marine Highway System port is located northwest of Auke Bay. The ABMS dock itself is relatively small and incorporates a building that stands upon some of the 48 creosote pilings located there. The dock at IPFO is just outside Auke Bay in Indian Cove near the entrance to Favorite Channel. The dock is composed of 97 creosote pilings. This dock is used solely by the National Park Service.

In October 2011, five replicate passive samplers were deployed and retrieved at all three sites at three distances of approximately: 10 cm, 1 m, and 10 m from a creosoted treated piling. Beginning in June of 2012 and continuing until October 3, three replicate passive samplers at the same distances and sites were deployed for two weeks once a month except for ABMS where deployment and retrieval occurred every two weeks. The deployment period for all samplers in this study was 14 days. At the docks at ABMS and IPFO, a rope was secured to a creosoted piling and wrapped around two others within a matrix of parallel pilings in two rows. From this rope, passive samplers were hung at 10 cm and 1 m from the piling. For the 10 m samples at ABMS and IPFO, passive samplers were hung from two crab pot buoys held in position by an

anchor. Four passive sampling strips were also attached directly to the surface of creosoted pilings at the ABMS by a thumb tack placed on the top and bottom of the sampling strip to measure hydrocarbons leaching from the surface of the piling. These were placed low enough on the piling such that they remained submerged. At AH, both 10 cm and 1 m samples were deployed within the confines of covered houseboat slips in order to minimize hydrocarbon signals from boat motors. The 10 cm samples were hung from metal rings surrounding the pilings, while 1 m samples were hung from floats approximately 1 m from creosoted pilings. Buoy deployment was not achieved at AH due to possible impediment of boat traffic; therefore 10 m samples were placed at the ends of float fingers closest to the harbor opening. In total, 203environmental field samples were collected.

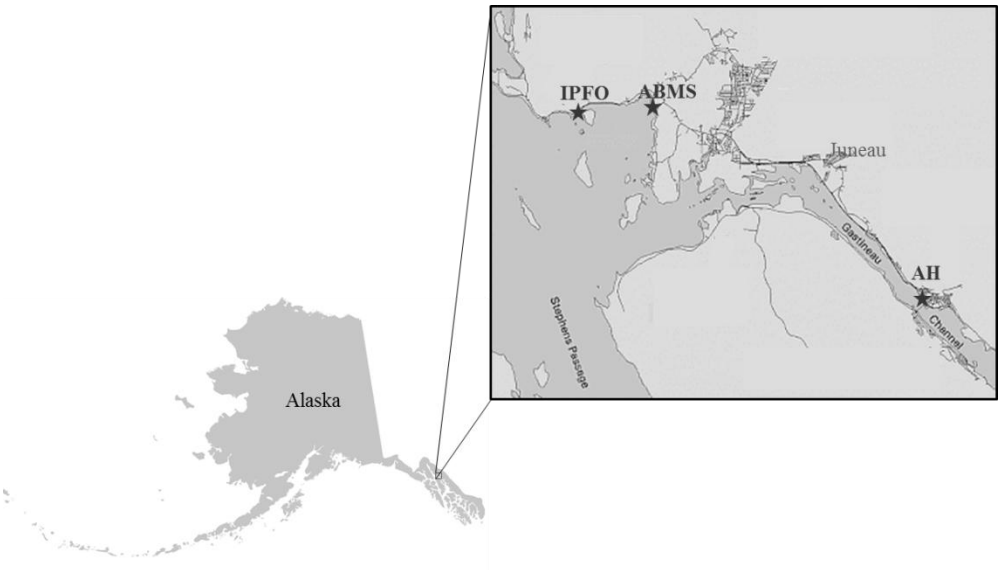

Fig. 2.1. Field site locations in Juneau, Alaska. IPFO: USFS Indian Point Field Office, ABMS: NOAA Auke Bay Marine Station, AH: Aurora Harbor. Modified from http://stickpaste.blogspot.com/2008_04_01_archive.html

Water current measurements and water sampling

Water current velocities were measured at each field site with a Marsh McBirney Flo-Mate Model 2000 portable flowmeter equipped with a telescoping aluminum/fiberglass pole (approximate max length –7 m) on June 8, 2012. Measurements were taken at three different times during the day at each location in order to measure variable tides. These measurements were taken at various depths from surface to bottom near pilings and on one occasion 10 m from pilings.

Although "snapshot" water samples may not be as effective as passive sampling for trace and/or intermittent PAH monitoring, three water samples (3.8 L) from each field site were collected during a low, mid, and high tide in mid-October of 2011 for a total of three samples at each site. Samples from the ABMS and AH were taken by submerging a 3.8 L hydrocarbon clean bottle from the docks. At the IPFO, the dock was closed, so samples were taken by walking into the water and submerging the bottle. These samples were spiked with deuterated PAH standards and extracted with methylene chloride and analyzed on a gas chromatograph equipped with a mass spectrometer as discussed above and in Carls et al. (2004).

Modeling and statistical analyses

R Studio version 0.94.110 statistical software was used for all statistical analyses. To compare TPAH concentrations in passive samplers, concentrations were modeled as a function of field site, distance, and date retrieved using ANOVA. Data were approximately normally distributed and variances were similar for most sets of observations. Concentrations were log-transformed prior to analysis. Results were considered significant if $p \leq 0.05$. Tukey contrasts were used to differentiate between variable levels. The effect of temperature on passive sampler TPAH concentration was modeled with linear regression.

PAH composition was analyzed by principle components analysis (PCA) using the prcomp function in R. Prior to analysis, individual component concentrations were converted into proportion values by dividing each by the total PAH, before unitizing such that the variance of each component was equal to one. Composition data from the passive samplers in this study

were compared to those from a prior laboratory toxicity study (Duncan 2014) and to reference data obtained from the NOAA Auke Bay Laboratory hydrocarbon database.

For direct comparisons between laboratory and field data, passive samplers used in the laboratory and in the field were manufactured with identical material and were prepared in the same way, although using two different sizes and were extracted and analyzed for PAHs by an identical method. They were deployed for the same amount of time, 14 days in seawater originating from Auke Bay though the time of year varied.

Aqueous TPAH concentrations from environmental passive sampler data were estimated using linear regression of passive sampler concentrations against associated mean water concentrations from data collected in the laboratory (Carls et al. 2004; Duncan 2014). By using similar published data, we were able to increase the range and data points of this regression. Prior to concentration modeling, passive sampler data were first standardized by dividing the TPAH value by the weight of the sampler (because smaller passive samplers were used in the laboratory), then dividing by the number of days deployed. This resulted in a linear, time integrated model that estimated the dissolved concentration of PAHs in water given passive sampler data.

RESULTS

PAH concentrations in blanks

PAHs were easily detected at all three sites and were several orders of magnitude (10-1000 times) above the blanks, indicating that our preparation and handling methods did not complicate interpretations. Blanks taken in the laboratory were very low in PAH; the average concentration for the 15 blanks taken in the laboratory during passive sampler preparation was 15 ng/g device (SE=1.5). Trip blanks (samplers never opened during site sampling events, but packed and transported with all other samples) were taken at each location at various points during the study. At each location blanks were taken between five and seven times. The average TPAH value for these was 120 ng/g device (SE=5.3). Field blanks (samplers both transported and briefly exposed to field conditions, to verify field cleanliness) were also taken at each location four times over the period and the average concentration for these was 220 ng/g device

(SE=14). The PAHs measured in the blanks were generally low molecular weight PAHs that are found virtually everywhere and are a testament to the passive sampler's affinity for hydrocarbons.

Field water velocities and PAH in water samples – Direct sampling

The average water velocity at the field locations on the date measured was 2 cm/sec. However, these measurements were below the certification limit of 15 cm/sec listed in the unit specifications. Observations of surface debris within 1-10 m of creosoted pilings suggested that current velocities were negligible. In addition, visible oil sheens (when present) around creosoted pilings had minimal movement.

PAH concentrations in "snapshot" water samples taken on three different tide intervals at each site were generally low, but above detection limits from the docks at both the ABMS and AH. These had mean TPAH concentrations of 0.33 (SE = 0.083) and 0.20 (SE = 0.0088) µg/L respectively. Samples taken by walking from the beach at the IPFO were higher, having a mean of 4.6 (SE=1.3) µg/L TPAH.

PAH concentrations in passive samplers

PAH concentrations in passive samplers varied widely between sites. AH had the least hydrocarbon loading of the three sites. Loadings at the IPFO were a factor greater than those at AH and those at the ABMS were a factor greater than those at the IPFO. However the TPAH concentrations measured at the ABMS and IPFO sites did not differ significantly, but both were significantly higher than those at AH. The highest concentrations of PAHs were measured at the ABMS (300,000 ng TPAH/g device). Passive samplers attached to the surface of creosoted pilings at the ABMS were even greater, between 50 and 400% greater than all other samples from the ABMS. TPAH concentrations at AH were significantly lower than those at the other two field sites and had the least variability ($p \leq 0.01$) (Fig. 2.2). The highest value measured at AH was 7000 ng/g; this is approximately two orders of magnitude less than at the ABMS. These smaller TPAH values measured at AH may be a result of less boating activity than at the other

two sites especially since the samplers were placed near houseboats which are not likely to be moved frequently.

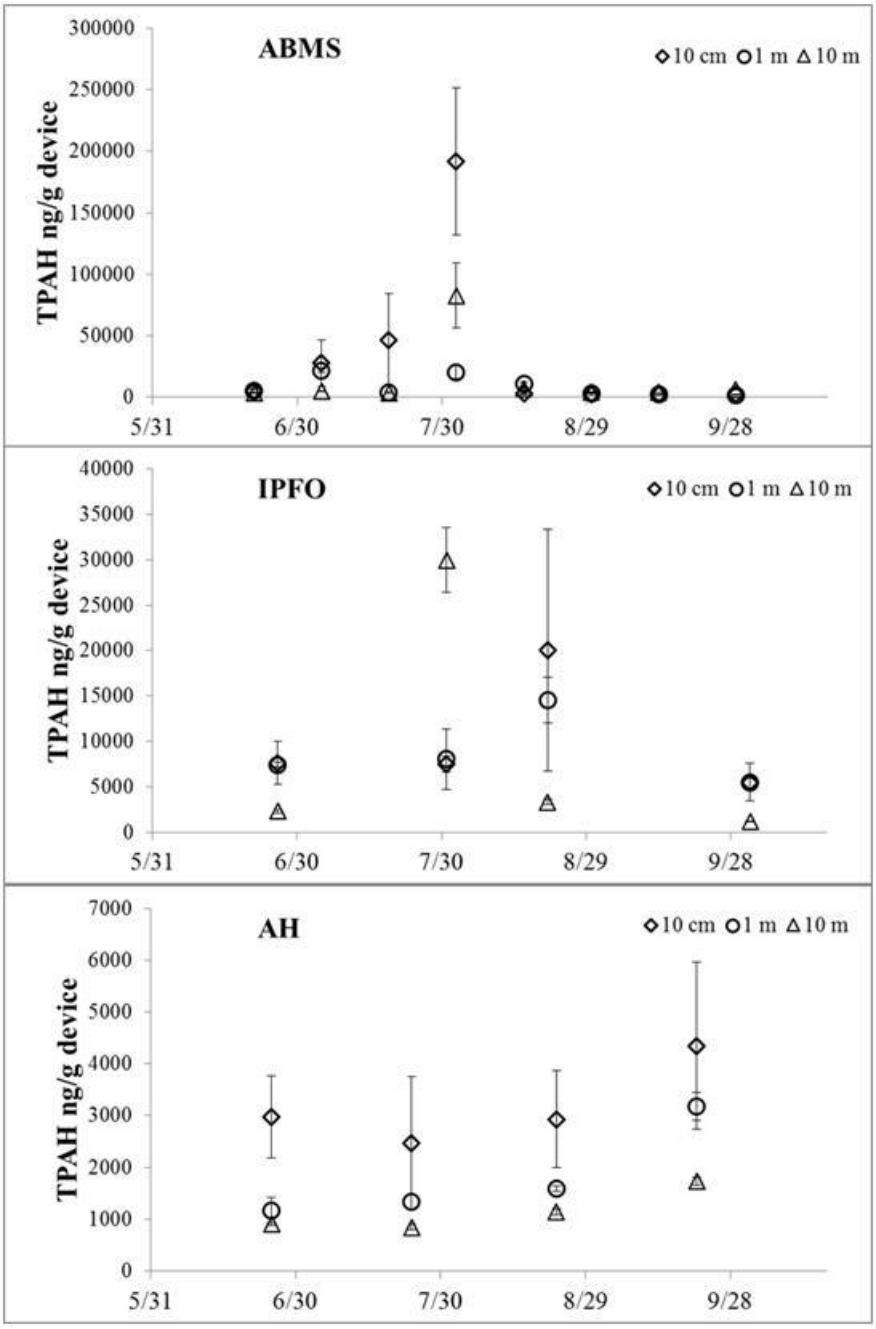

Fig. 2.2. TPAH concentrations in passive samplers (means ± SE). Sample were collected in the spring and summer of 2012. Sites from top to bottom: Auke Bay Marine Station, Indian Point Field Office, and Aurora Harbor. Note different y axis scales.

PAH concentrations as a function of distance from creosoted pilings

Creosote concentrations varied as a function of distance from creosoted pilings at AH and were highest within 10 cm suggesting that the creosote pilings may be influential. TPAH concentrations were significantly greater at 10 cm and 1 m from creosoted pilings than at the 10 m points at the ends of float fingers near the harbor opening ($p = 0.00018$ and 0.046 respectively, Fig. 2.3). Differences in TPAH concentration as a function of distance from creosoted pilings were not detected at the ABMS or the IPFO field sites, although when there was some spread in the values, the highest ones were often the 10 cm samples ($p \leq 0.10$).

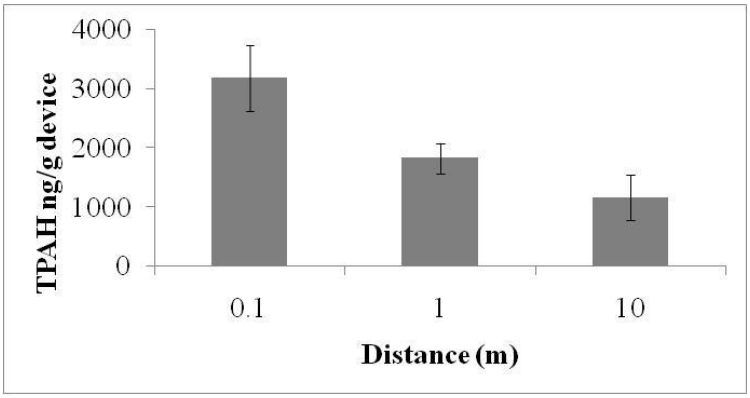

Fig. 2.3. TPAH as a function of distance from creosoted pilings at AH. TPAH concentrations were significantly greater at 10 cm and 1 m from pilings than at 10 m. ($p = 0.00018$ and 0.046 respectively) (means± SE).

Seasonal spike in TPAH concentrations

Seasonal spikes in hydrocarbon concentrations were observed in July at the ABMS and the IPFO site, while AH had more constant concentrations over time. The seasonal spike in hydrocarbon concentrations at the ABMS and IPFO sites displayed significantly higher TPAH concentrations in late July-early August ($p \leq 0.05$, Fig. 2.4). Given the apparent effect of date on TPAH concentration, temperature was also explored as an effect using the larger data set collected at the ABMS. Temperature data for ABMS was acquired from the NOAA Auke Bay Laboratories Ted Stevens Marine Research Institute. Temperature means for the deployment

period were plotted and regressed versus TPAH concentration for all three distances from creosoted pilings. TPAH concentrations in passive samplers showed a weak positive correlation with temperature ($p \leq 0.03$, $R^2 = 0.47$, Fig. 2.4). Boating activity also increased (anecdotal observations) as the summer progressed, but there was no quantitative data on boat activity available.

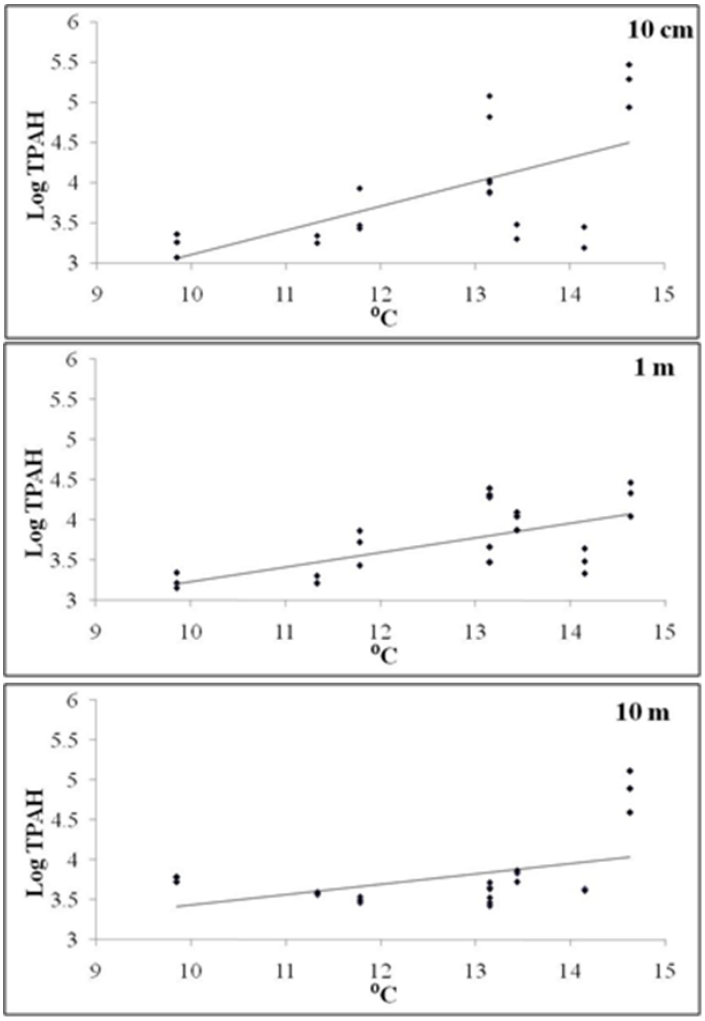

Fig. 2.4. TPAH concentrations as a function of water temperature. The increase of TPAH with temperature was most apparent for samples located 1 m from creosoted pilings ($R^2 = 0.41$, $p \leq 0.03$), however 10 cm and 10 m samples were similarly affected ($R^2 = 0.38$ and 0.20 respectively).

Comparison of passive sampler data from the field and the laboratory

Direct comparisons of laboratory and field data identified eight cases where the laboratory established NOEC (no observed effect concentration) for skeletal defects was met or exceeded. This value, 4.3 µg/L, was correlated with a passive sampler value of 44,000 ng TPAH/g device over a 14 day period in a previous laboratory experiment. The LOEC (lowest observed effect concentration) for skeletal defects (6.8 µg/L mean TPAH over a 14 day period) was correlated with a passive sampler value of 81,000 ng TPAH/g device. Given this information, the true effect concentration is somewhere in between these values and the NOEC was used for comparisons with field data. The NOEC value was met or exceeded in eight passive samples, primarily at the ABMS site and within 10 cm from a creosoted piling. In one case, also at the ABMS, the NOEC was exceeded at 10 m from a creosoted piling. The highest TPAH value recorded was 290,000 ng TPAH/g device at a distance of 10 cm at the ABMS. Although not directly comparable because the measured hydrocarbons may or may not have been in solution, samples located on the surface of creosoted pilings ranged from 130,000 to 390,000 ng TPAH/g device.

An estimation of the actual water concentrations of PAHs by using correlations determined in the laboratory

Ambient water concentrations were estimated from field passive sampler data with the following model: $y = 0.0017x + 0$, where y = the estimated mean daily TPAH concentration in the water over the deployment period, and x= (ng TPAH/g device)/days deployed, forced through zero, $p \leq 0.05$, $R^2 = 0.91$. This model was used to estimate TPAH concentrations in seawater over the deployment period for the field sites (Fig. 2.5). The results of this model with respect to the laboratory-determined NOEC and LOEC were similar to the direct comparisons previously described. Fourteen (6%) of the field samples had estimated dissolved TPAH concentrations greater than or equal to the NOEC, and 5% of these were above the LOEC. The range for these samples was 4-36 µg/L TPAH, and the majority of these were located within 10 cm from a creosoted piling. Apart from these elevated concentrations, the majority of samples

(68%) taken from field sites at differing distances from creosoted pilings had estimated dissolved TPAH concentrations < 1 µg/L according to the model.

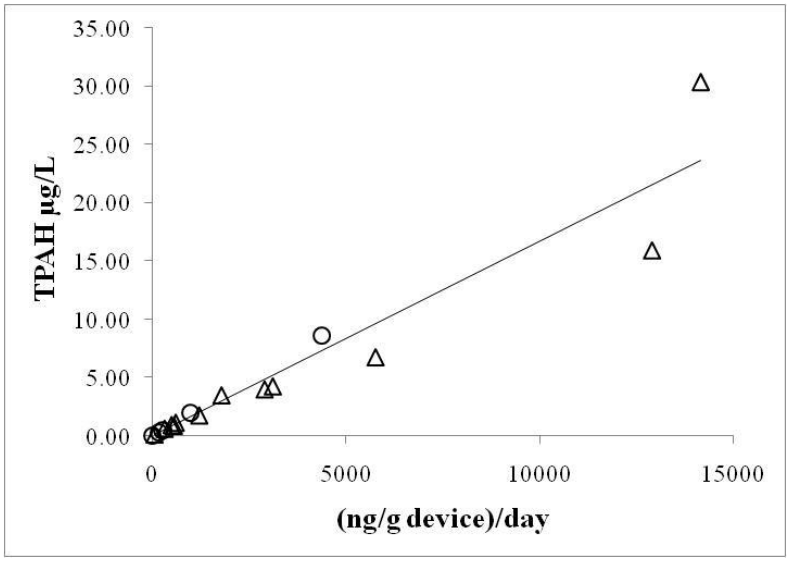

Fig. 2.5 Correlation of TPAH concentrations in water and passive samplers. Data pooled from previous creosote laboratory experiment (triangles) and Carls et al. 2004 (circles). $R^2 = 0.91$, $p \leq 0.05$.

PAH composition of environmental samples

Qualitatively, the PAH compositions of the environmental samples appeared to be of both pyrogenic and petrogenic sources, but the vast majority were dominated by unsubstituted high molecular weight PAHs (HPAH), which indicates mostly pyrogenic sources such as creosote (Wang et al 2008). In the cumulative PCA plot of all three sites combined with historic data (Fig. 2.6), samples located to the right of the midpoint or center (x=2.5) of the x axis had a higher proportion of substituted low molecular weight PAH (LPAH) than HPAH, while samples to the left had a higher proportion of unsubstituted HPAH. The field sites were roughly located near each other on the PCA plot, suggesting similar PAH compositions. In all but six cases, the field samples and laboratory creosote samples were distributed to the left of center on the x axis and closer to each other than to Alaska North Slope crude oil (a distinctly petrogenic source), again indicating primarily pyrogenic sources. The laboratory creosote samples were distributed

within the same space that some samples from all three field sites occupied, again suggesting similar compositions. In addition, some samples on the surface of creosoted pilings at the ABMS were co-located with samples from the ABMS and IPFO suggesting a common source.

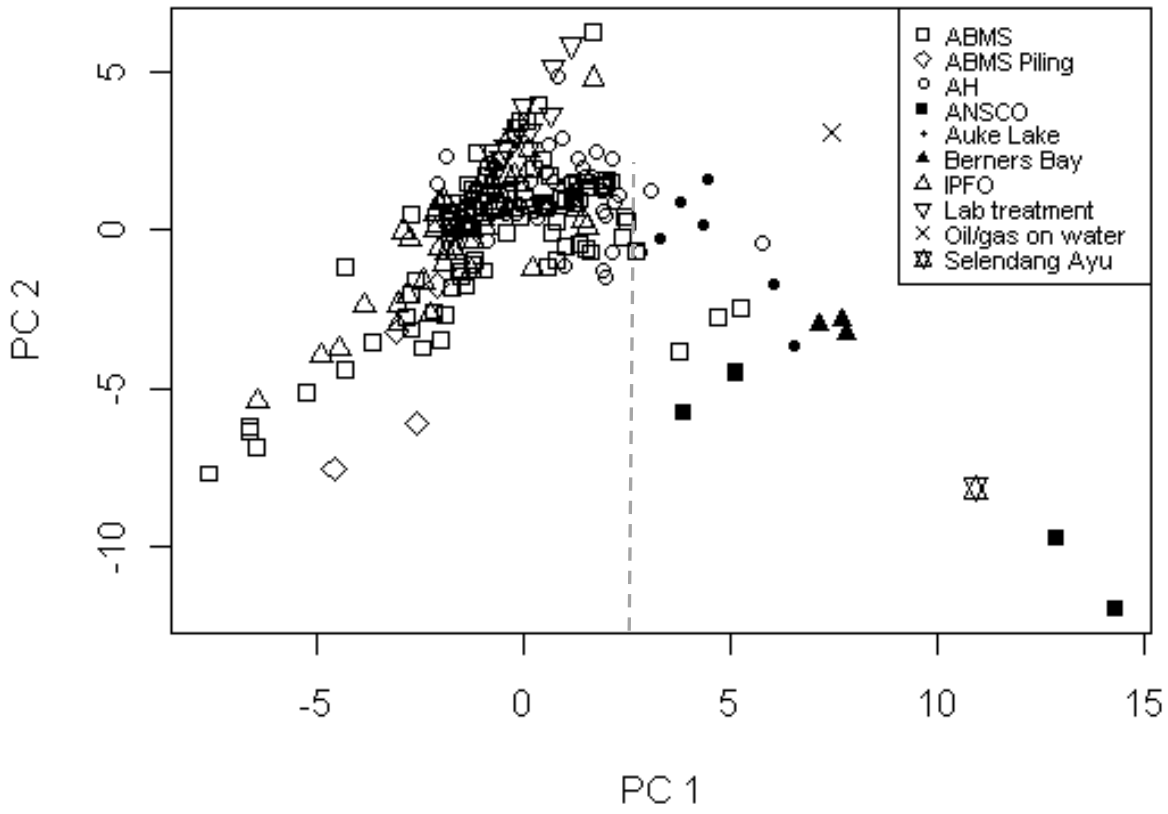

Fig. 2.6. Cumulative PCA plot of passive sampler PAH composition data. Data from the three field sites and lab (open symbols) plus historic samples indicated similar and generally pyrogenic composition. Dashed line is putative dividing line with pyrogenic type PAHs on the left and petrogenic type PAHs on the right. Legend from top to bottom: Auke Bay Marine Station, Auke Bay Marine Station creosoted piling, Aurora Harbor, Alaska North Slope Crude Oil, Auke Lake, Berners Bay, Indian Point Field Office, lab creosote treatment, oil/gas on water, and Selendang Ayu oil.

PAH composition as a function of distance from a creosoted piling

In some cases, the composition of PAHs varied depending on distance from creosoted pilings. These effects were most apparent in samples from AH where TPAH concentrations

were much lower than at the other two sites making source detection less difficult. At AH the PCA plot of the distance data alone, without reference samples, indicated a gradual PAH composition change from 10 cm to 10 m (Fig. 2.7). Differences in composition as a function of distance from creosoted pilings at IPFO were not clearly defined but were suggestive of a gradual composition gradient from 10 cm to 10 m (Fig. 2.7). At the ABMS, 1 m and 10 m samples appeared somewhat distinct from each other, while 10 cm samples seemed distributed among both distances (Fig. 2.7).

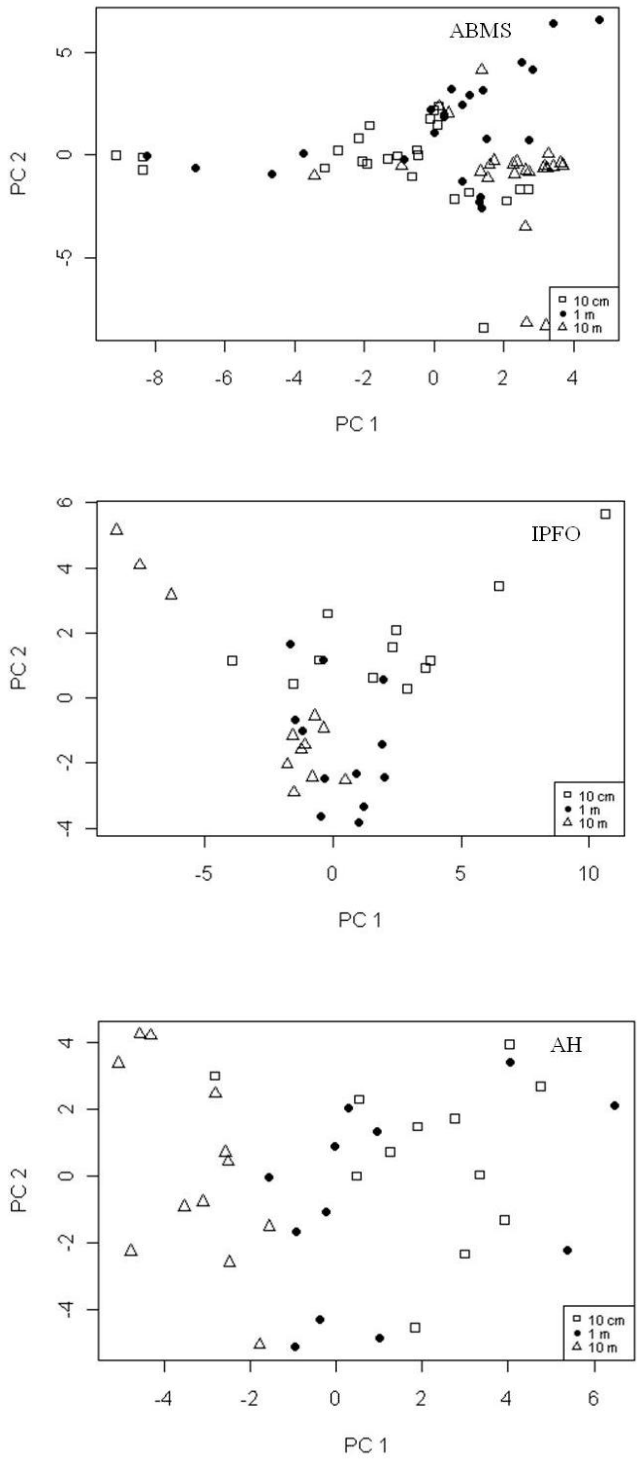

Fig. 2.7. PCA plot of passive sampler PAH composition near creosoted pilings. Samplers were located 10 cm, 1 m, and 10 m from a creosoted piling at each of three field sites (Auke Bay Marine Station, Indian Point Field Office, and Aurora Harbor). There was some evidence of a PAH compositional change as a function of distance at Aurora Harbor.

DISCUSSION

Increased hydrocarbon loading closest to creosoted pilings was observed in some cases. Increased PAH concentrations as a function of distance from creosoted pilings were most evident at AH where concentrations in general were lower. At AH, TPAH concentrations were significantly elevated 10 cm and 1 m away from creosoted pilings compared to near the harbor opening. There was some evidence that the composition of PAHs shifted with distance at AH. No significant effect of distance from creosoted pilings on PAH concentration was observed for the other two field sites, both of which had significantly greater concentrations of hydrocarbons than AH. Hydrocarbon loading in seawater as a function of distance from a creosoted piling may have been confounded by seasonal increases in other hydrocarbon inputs at the other two sites. Higher temperatures have two significant effects: first they likely increase the leaching and diffusion of creosote PAHs from the pilings, and second, boating activity, particularly outboard use, increases dramatically in the summer, thus confounding and possibly masking the creosote contributions. A similar study conducted in Auke Lake Juneau, Alaska, found a correlation between monthly PAH increases and the amount of watercraft using the lake (Rice et al. 2008).

Temperature was correlated with TPAH concentration in samplers independent of distance from pilings and dates sampled at the ABMS where more data were available. Temperature directly affects PAH solubility which varies widely as a function of the individual compounds. In general, PAH solubility decreases as molecular mass and salinity increase, and increases with increasing temperature (Neff 2002). For example, a high molecular weight PAH such as dibenz[a,h]anthracene may have an aqueous solubility of 0.6 µg/L whereas a low molecular weight PAH such as naphthalene would have an aqueous solubility of 31,500 µg/L under the same environmental conditions. Although creosote is not very water soluble, the PAHs can dissolve and/or diffuse into surrounding water and become mobile and bioavailable. Once in solution, they may be adsorbed onto particles or sediment depending on temperature and salinity (Neff 2002).

Numerous studies have demonstrated PAH toxicity in developing fish embryos at concentrations as low as 0.4 to 23 µg/L TPAH (Carls et al. 1999; Hershberger et al. 2005, 2008; Incardona et al. 2009). The passive samplers near pilings were close to, and some were above, this concentration correlating with PAH concentrations in the µg/L range. Creosote is toxic to

Pacific herring at TPAH concentrations in the range of 5-50 µg/L (Vines et al. 2000; Duncan 2014). Creosote exposure at this low level can result in mortality, skeletal defects, and ineffective swimming. Trace levels, (<1 µg TPAH/L) can bioaccumulate in fish embryos and may have population level effects (Short et al. 2003). Pacific herring sometimes spawn directly on or near creosoted structures. They are sensitive to shoreline development because their eggs are spawned nearshore where they develop, hatch, and grow until they are large enough to migrate off shore (Lassuy 1989; Leet et al. 2001; Penttila 2007; Werme et al. 2010).

The field data collected in this study can also be compared to a passive sampling study conducted following the *Selendang Ayu* oil and soybean spill in 2004 that used identical equipment and methodology. In that study, the authors identified oiled streams that had passive sampler TPAH values that exceeded 100,000 ng/g device, (with an estimated water concentration of 2 µg/L), a probable risk to developing embryos and juvenile fish (Carls et al. 2008). For comparison, the current study measured a maximum TPAH value 10 cm from a creosoted piling of 290,000 ng/g device over a 14 day period, nearly three times the concentration and deployed for less than half as long. Therefore, it is likely that passive sampler concentrations in this study that were deployed for less time, and had concentrations ≥ 100,000 ng/g device are representative of concentrations of PAHs in the water likely to be a hazard to herring embryos and possibly other organisms.

This study documented significant aqueous concentrations of PAHs in harbors in a subarctic marine ecosystem at temperatures that ranged from approximately 9-15 °C and identified cases where the PAH composition in passive samplers was consistent with creosote as the source. However, it cannot be absolutely certain that the aqueous PAHs in the field were originating from the creosote pilings themselves or the environment around them such as the sediment (a possible point-source of creosote contamination). Other sources were likely contributors of PAHs, particularly in the summer when boating activity was probably increased. The AH sampling, with the lowest concentrations of PAH, were the best evidence of creosote as a primary source, based on decreasing PAH concentrations with distance from pilings.

The concentrations of PAHs in the harbors approach significantly toxic concentrations, no matter what the specific sources are. Wave and tidal action on creosoted pilings and the hardware around them, such as metal rings, can cause rubbing and subsequent release of fresh creosote into the surrounding water and sediment. Hydrocarbon contamination in harbors and

marinas is not uncommon. Both AH and the Auke Bay Marina have documented hydrocarbon contamination in sediments on the order of 4-5 µg/g (dry weight) (Ziemann et al. 1990). Because PAH compositions indicative of creosote occurred more often at 10 cm and 1 m from creosoted structures, and because the water current data suggest that relatively low current velocity at our field sites may keep PAHs close to where they originated, it is likely that some of the PAHs near the pilings originate from the pilings themselves. A similar study investigating creosote contaminated sediments in a marina had comparable results. Evans et al. (2009) detected high concentrations of PAHs in sediments near pilings and a marked decrease in PAHs two meters from pilings. The authors also noted that without sufficient wave action and water currents in protected marinas, PAHs may remain in the water column next to pilings. This phenomenon may be common for docks or harbors located in waters with slow or negligible currents, such as sheltered bays or coves. In a study on Alaska North Slope crude oil toxicity to embryonic Pacific herring (*Clupea pallasi*), both passive sampler and tissue PAH concentrations were correlated with initial water concentrations. The PAH compositions in the two matrices, though not exactly identical, were also related (Carls et al. 2004). A similar experiment concurrently exposing polychaetes (*Nereis virens*) and passive samplers to contaminated sediments from Boston Harbor observed the same relationship between TPAH concentration and composition in organisms and in passive samplers (Vinturella et al. 2004).

This study found instances where dissolved PAHs near creosoted pilings are likely sufficient to cause teratogenic effects on Pacific herring embryos. Some of this PAH leaches from the piles and direct spawning on creosoted pilings would have a higher risk of teratogenic effects than spawning away from the pilings. Deposition of eggs on biofouling organisms or structures near pilings would also have an increased risk. Direct concentration comparisons with field and laboratory data from passive samplers identified occasions where PAH concentrations were greater than or equal to the laboratory established LOEC of 81,000 ng TPAH/g device, equal to approximately 6.8 µg/L mean concentration over the course of 14 days. These concentrations occurred within 10 cm and in one case 10 m from a creosoted piling and indicate that creosote may contribute to hydrocarbon pollution near pilings and could negatively impact developing herring and other embryos in the nearshore environment.

References

Adams RG, Lohmann R, Fernandez LA, MacFarlane JK, Gschwend PM. 2007. Polyethylene devices: Passive samplers for measuring dissolved hydrophobic compounds in aquatic environments. *Environ Sci Technol* 41:1317–1323.

ADFG (Alaska Department of Fish and Game). Divisions of Sport Fish and Commercial Fisheries. 2005. Special Publication No. 05-09 Commercial Fisheries of Alaska, Anchorage, AK. [cited 30 November 2013]. Available from: http://www.adfg.alaska.gov/FedAidPDFs/sp05-09.pdf. 9-17.

Alderdice DF, Hourston AS. 1985. Factors influencing development and survival of Pacific herring (*Clupea harengus pallasi*) eggs and larvae to beginning of exogenous feeding. *Can J Fish AquatSci*42:56-68.

Allan IJ, Harman C, Kringstad A, Bratsberg E. 2010. Effect of sampler material on the uptake of PAHs into passive sampling devices. *Chemosphere* 79:470-475.

Allan, SE, Sower, GJ, Anderson, KA. 2011. Estimating risk at a Superfund site using passive sampling devices as biological surrogates in human health risk models. *Chemosphere* 85:920-927.

Anderson KA, Sethajintanin D, Sower G, Quarles L. 2008. Field trial and modeling of uptake rates of in situ lipid-free polyethylene membrane passive sampler. *Environ Sci Technol* 42:4486-4493.

Bao, L. J., Xu, S. P., Liang, Y., Zeng, E. Y. 2012. Development of a low-density polyethylene-containing passive sampler for measuring dissolved hydrophobic organic compounds in open waters. *Environ Toxicol Chem* 31: 1012-1018.

Carls MG, Rice SD, Hose JE. 1999. Sensitivity of fish embryos to weathered crude oil: Part 1. Low-level exposure during incubation causes malformations, genetic damage, and mortality in larval Pacific herring (*Clupea pallasi*). *Environ Toxicol Chem* 18:481-493.

Carls MG, Holland LG, Short JW, Heintz RA, Rice SD. 2004. Monitoring polynuclear aromatic hydrocarbons in aqueous environments with passive low-density polyethylene membrane devices. *Environ Toxicol Chem* 23:1416-1424.

Carls MG, Hudson J, Rice SD.2008.SelendangAyu oil risk to early life stage salmon.Auke Bay Laboratories, Alaska Fisheries Science Center, NOAA Fisheries. Juneau, AK. 1-23.

CBJ (City and Borough of Juneau) Docks and Harbors and PND Engineers Inc. 2013. Aurora Harbor rebuildmasterplan. Juneau, AK. 1-88.

Duncan DL. 2014. The toxicity of creosote treated wood to Pacific herring (*Clupea pallasi*) embryos and characterization of polycyclic aromatic hydrocarbons in Juneau, Alaska. (Unpublished masters thesis). University of Alaska Fairbanks, Fairbanks, Alaska.

Evans MS, Fazakas K, Keating J. 2009. Creosote contamination in sediments of the Grey Owl Marina in Prince Albert National Park, Saskatchewan, Canada. *Water, Air, Soil Pollut* 201:161-184.

Griffin FJ, Smith EH, Vines CA, Cherr GN. 2009. Impacts of suspended sediments on fertilization, embryonic development, and early larval life stages of the Pacific herring, *Clupea pallasi*. *Biol Bull* 216:175-187.

Haegele CW, Schweigert JF. 1985. Distribution and characteristics of herring spawning grounds and description of spawning behavior. *Can J Fish Aquat Sci* 42 (Suppl. 1):39-55.

Hershberger PK, Elder NE, Wittouck J, Stick K, Kocan RM. 2005. Abnormalities in larvae from the once-largest Pacific herring population in Washington state result primarily from factors independent of spawning location. *Trans Am Fish Soc* 134:326-337.

Incardona J, Carls MG, Day HL, Sloan CA, Bolton JL, Collier TK, Scholz NL. 2009. Cardiac arrhythmia is the primary response of embryonic Pacific herring (*Clupea pallasi*) exposed to crude oil during weathering. *Environ Sci Technol* 43: 201-207.

Lassuy DR. 1989. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (Pacific Northwest). Report of Oregon Cooperative Fishery Research Unit to U.S. Army Corps of Engineers Coastal Ecology Group Waterways Experiment Station Vicksburg, LA and U.S. Department of the Interior Fish and Wildlife Service, Washington, DC. [cited 1 July 2014]. http://www.nwrc.usgs.gov/wdb/pub/species_profiles/82_11-126.pdf. 1-14.

Leet WS, Dewees CM, Klingbeil R, Larson EJ, editors. 2001. California's living marine resources: A status report. California Department of Fish and Game Resources Agency.456-459.

McGurk MD. 1989. Early life history of Pacific herring in Auke Bay, Alaska: relationships of growth and survival to environmental conditions. Final Report.1-289.

Neff JM. 2002. Bioaccumulation in marine organisms: effect of contaminants from oil well produced water. Elsevier Inc., San Diego, California, USA. 241-314.

O'Farrell MR. Larson RJ. 2005. Year-class formation in Pacific herring (*Clupea pallasi*) estimated from spawning-date distributions of juveniles in San Francisco Bay, California. Fish Bull 103:130-141.

Penttila, D. 2007. Technical Report 2007-3 Marine forage fishes in Puget Sound. Washington Department of Fish and Wildlife in support of Puget Sound Nearshore Partnership, Olympia, Washington.[cited 2 July 2014]. Available from: http://www.pugetsoundnearshore.org/technical_papers/marine_fish.pdf 1-14. 1-20.

Rice SD, Holland L, Moles A. 2008. Seasonal increases in polycyclic aromatic hydrocarbons related to two-stroke engine use in a small Alaskan lake. *Lake Reservoir Manage* 24:10-17.

Short JW, Jackson TJ, Larsen ML, Wade TL. 1996. Analytical methods used for the analysis of hydrocarbons in crude oil, tissues, sediments, and seawater collected for the natural resources damage assessment of the *Exxon Valdez* oil spill. *Am Fish Soc Symp* 18:140–148.

Short JW, Rice SD, Heintz RA, Carls MG, Moles A. 2003. Long-term effects of crude oil on developing fish: lessons from the *Exxon Valdez* oil spill. *Energy Sources* 25:509-517.

Small MP, Loxterman JL, Frye AE, Von Bargen JF, Bowman C, Young SF. 2005. Temporal and spatial genetic structure among some Pacific herring populations in Puget Sound and the southern Strait of Georgia *Trans Am Fish Soc* 134:1329-1341.

Vines CA, Robbins T, Griffin FJ, Cherr GN. 2000. The effects of diffusible creosote-derived compounds on development in Pacific herring (*Clupea pallasi*). *Aquat Toxicol* 51:225-239.

Vinturella AE, Burgess RM, Coull BA, Thompson KM, Shine JP. 2004. Use of passive samplers to mimic uptake of polycyclic aromatic hydrocarbons by benthic Polychaetes. *Environ Sci Technol* 38: 1154-1160.

Wang Z, Yang C, Brown C, Hollebone B, Landriault M. 2008. A case study: distinguishing pyrogenic hydrocarbons from petrogenic hydrocarbons. International Oil Spill Conference Proceedings, May 2008, Vol. 2008, No. 1:311-320.

Werme C, Hunt J, Beller E, Cayce K, Klatt M, Melwani A, Polson E, Grossinger R. 2010. San Francisco Bay subtidal habitat goals report. Appendix 6-1: Removal of creosote treated pilings and structures from San Francisco Bay.[cited 30 November 2013]. Available from: http://www.sfbaysubtidal.org/PDFS/Ap6-1%20Creosote-Artificial%20Structures.pdf. 1-47.

Ziemann DA, Fulton-Bennett KW. Editors. 1990. APPRISE- Interannual variability and fisheries recruitment. The Oceanic Institute. Honolulu, HI. 1-416.

GENERAL CONCLUSIONS

This thesis and the manuscripts therein contribute to a growing body of scientific evidence that creosote treated wood is toxic to Pacific herring embryos and is a lasting point source of PAHs. Chapter One determined that creosote treated wood is toxic to developing Pacific herring embryos at concentrations up to ten times lower than previously documented (7 µg/L TPAH). Significant concentrations of PAHs leaching from the creosoted wood were measured in tissues of developing embryos. Creosote exposure resulted in increased mortality rates in addition to skeletal defects and retarded swimming ability. These effects can have population-level consequences (Short et al. 2003). Chapter Two of this thesis compared the toxicological data gathered in the laboratory with environmental data collected at three harbor sites in Juneau, AK and found that PAH concentrations sufficient to induce toxic effects exist both in direct contact with pilings and within 10 cm. These elevated concentrations occurred most frequently within 10 cm from pilings and during late summer when water temperatures and boating activity were highest. Composition analysis of the environmental samples indicated that the vast majority of PAHs were from pyrogenic sources, in some cases, consistent with creosote. However, at the field sites with the highest concentrations of aqueous PAHs, there were mixed sources and increased loading may have confounded the impact of creosote.

These data can be used by both engineers and biologists to better understand the effect of using creosote treated wood for structures in the nearshore marine environment where herring spawn. The results of Chapter One have already been shared with the State of Alaska Department of Transportation for use in their decision making process for docks and harbors. Both chapters will be submitted to the Journal of Environmental Toxicology and Chemistry for publication.

Future studies are needed on the toxicity to Pacific herring embryos of other nearshore building material options such as concrete, steel, and copper treated pilings. In addition, little data exists regarding the effects (both positive and negative) of nearshore structures on marine habitat. Non-point and point source pollution effects on nearshore organisms in urbanized areas are also lacking current data. Passive environmental sampling both for quantification and source identification are areas that need further research in order to standardize data for regulatory use in monitoring and characterizing pollution events.

References

Short JW, Rice SD, Heintz RA, Carls MG, Moles A. 2003. Long-term effects of crude oil on developing fish: lessons from the *Exxon Valdez* oil spill. *Energy Sources* 25:509-517.

Appendix: IACUC Approval

(907) 474-7800
(907) 474-5638 fax
fyiacuc@uaf.edu
www.uaf.edu/iacuc

Institutional Animal Care and Use Committee
909 N Koyukuk Dr. Suite 212, P.O. Box 757270, Fairbanks, Alaska 99775-7270

March 13, 2012

To:	Michael Stekoll Principal Investigator
From:	University of Alaska Fairbanks IACUC
Re:	[210243-4] Mortality and physiological responses of Pacific herring (Clupea pallasi) embryos to varying concentrations of creosote treated wood in a flowing marine laboratory environment.

The IACUC has reviewed the Progress Report by Designated Member Review and the Protocol has been approved for an additional year.

Received:	March 7, 2012
Initial Approval Date:	April 4, 2011
Effective Date:	March 12, 2012
Expiration Date:	April 4, 2013

This action is included on the March 27, 2012 IACUC Agenda.

If you have any questions about how to submit the required information through IRBNet please contact the Office of Research Integrity for assistance (email fyori@uaf.edu or call x7800/x7832).